UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

012000

JUL 26 1996

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: **DICAMBA:** Review of Mutagenicity Studies with the **Dimethylamine (DMA), Diglycolamine (DGA) and Isopropylamine (IPA) Salts of Dicamba.**

FROM: Jess Rowland, M.S., Toxicologist Jess Rowland 7/24/96
Section I, Toxicology Branch II, Health Effects Division (7509C)

TO: Walter Waldrop / Jane Mitchell
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THRU: Yiannakis Ioannou, Ph.D, Head J.M. Ioannou 7/24/96
Section I, Toxicology Branch II, Health Effects Division (7509C)
and
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Toxicology Branch II, Health Effects Division J.M. Ioannou for 7/24/96

DATA PACKAGE IDENTIFICATION: Submission: S470884 DP Bardcode: D206005

Chemical	PC Code	Tox.Chem.No.	MRID No(s).
Dicamba-DMA	029802	**295B**	43310301 & 43310304
Dicamba-DGA	128931	**295F**	43310302 & 43310305
Dicamba-IPA	128944	**295G**	43310303 & 43310306

ACTION REQUESTED: Review six mutagenicity studies with the DMA, DGA and IPA salts of dicamba submitted by Sandoz Inc, to fulfill Subdivision F guideline requirements §84-2(a,b).

RESPONSE: Data Evaluation Records (DERs) for the six mutagenicity studies referenced above are attached. The Executive Summaries are presented below. When tested in the *Salmonella*/Microsome reverse mutation assay and in an *in vitro* mouse lymphoma assay, the DMA, DGA and IPA salts of dicamba were non mutagenic in both types of assays either in the presence or absence of metabolic activation.

All six studies are Core classified as acceptable and satisfy the Subdivision F guideline requirement (§84-2a,b) for *in vitro* mutagenicity assays.

I. DIMETHYLAMINE Salt of Dicamba (Banvel®) (MRID 43310301)

1. Ames Assay

CITATION: San, R., and D. Pugh (1994) *Salmonella* Plate Incorporation Mutagenicity Assay (Ames Test) with Confirmatory Assay. Microbiological Associates, Inc., Rockville, MD. Study # TE236.501014. 6/29/94. MRID 43310301. Unpublished.

EXECUTIVE SUMMARY: In a microbial mutagenicity assay (MRID 43310301), *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, or TA1538 were exposed to the DMA salt of dicamba (40.3% a.i.) in deionized distilled water at concentrations of 100, 333, 1000, 3333, or 5000 μg/plate in the presence and absence of mammalian metabolic activation. Preparations for metabolic activation were made from induced rat livers. The DMA salt of dicamba was tested up to the limit concentration of 5000 μg/plate and no cytotoxicity was observed. The positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background (reversion to prototrophy).**

This study is classified as **acceptable**, and satisfies the Subdivision F Guideline requirement §84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

2. *In Vitro* Mouse Lymphoma Assay (MRID No. 43310304)

CITATION: San, R., and J. Clarke. (1994) L5178Y/TK$^{+/-}$ Mouse lymphoma mutagenesis assay with a confirmatory assay. Microbiological Associates, Inc., Rockville, MD. Study # TE236.701020. 6/21/94. MRID No. 43310304. Unpublished.

EXECUTIVE SUMMARY: In a mammalian cell gene mutation assay at the thymidine kinase locus (MRID 43310304), L5178Y mouse lymphoma cells cultured *in vitro* were exposed to dicamba DMA salt (40.3% ai) in distilled water at concentrations of 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, and 5000 μg/mL in the presence and absence of S9 mammalian metabolic activation. Dicamba DMA salt was tested up to the limit dose. Under nonactivation conditions, the % total growth values over the evaluated dose range were from 69-109% (initial assay) and 65-111% (confirmatory assay). The mutation frequencies (MFs) for all of the treated cultures were <2x the solvent controls; the exception was the 4500 μg/mL dose, which had a MF of approximately 2x background in the confirmatory trial. However, the 4500 μg/mL response was not reproducible. The S9-activation assay confirmed the findings of the nonactivation assay. The % total growth values were 26-109% (initial assay) and 23-113% (confirmatory assay). The MFs for all of the treated cultures were <2x the solvent controls with the exception of the 3000 μg/mL dose in the confirmatory trial which had a MF of approximately 2x background; this result was not reproducible. It was determined that **dicamba DMA salt was not mutagenic under either nonactivation or S9-activation conditions**. In both the nonactivated and activated conditions, the positive controls induced the appropriate response.

This study is classified as **acceptable** and satisfies the Subdivision F Guideline requirement §84-2 for *in vitro* mutagenicity (mammalian forward gene mutation) data .

II. DIGLYCOLAMINE Salt of Dicamba

3. Ames Assay (MRID 43310302)

CITATION: San, R., and D. Pugh (1994) *Salmonella* Plate Incorporation Mutagenicity Assay (Ames Test) with Confirmatory Assay. Microbiological Associates, Inc., Rockville, MD. Study # TE237.501014. 6/29/94. MRID 43310302. Unpublished.

EXECUTIVE SUMMARY: In a microbial mutagenicity assay (MRID 43310302), *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, or TA1538 were exposed to the DGA salt of dicamba (39.7% a.i.) in deionized distilled water at concentrations of 100, 333, 1000, 3333, or 5000 μg/plate in the presence and absence of mammalian metabolic activation. Preparations for metabolic activation were made from induced rat livers. The DGA salt of dicamba was tested up to the limit concentration of 5000 μg/plate, but no cytotoxicity was observed. The positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background (reversion to prototrophy).**

This study is classified as **acceptable**, and satisfies the Subdivision F Guideline requirement §84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

4. *In Vitro* Mouse Lymphoma Assay (MRID No. 43310305)

CITATION: San, R., and J. Clarke. (1994) L5178Y/TK$^{+/-}$ Mouse lymphoma mutagenesis assay with a confirmatory assay. Microbiological Associates, Inc., Rockville, MD. Study # TE237.701020. 6/15/94. MRID 43310305. Unpublished.

EXECUTIVE SUMMARY: In a mammalian cell gene mutation assay at the thymidine kinase locus (MRID 43310305), L5178Y mouse lymphoma cells cultured *in vitro* were exposed to dicamba DGA salt (39.7% ai) in distilled water at concentrations of 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, and 5000 μg/mL in the presence and absence of S9 mammalian metabolic activation. Dicamba DGA salt was tested up to the limit dose. Under nonactivation conditions, the % total growth values over the evaluated dose range were from 68-116% (initial assay) and 72-105% (confirmatory assay). The mutation frequencies (MFs) for all of the treated cultures were $<2x$ the solvent controls. The S9-activation assay confirmed the findings of the nonactivation assay. The % total growth values were 43-102% (initial assay) and 46-99% (confirmatory assay). The MFs for all of the treated cultures were $<2x$ the solvent controls with the exception of the 4500 μg/mL dose in the initial trial, which had a MF of approximately 2x background. However, this result was not reproducible. Therefore, it was determined that **dicamba DGA salt was not mutagenic under nonactivation or S9-activation conditions.** In both the nonactivated and activated conditions, the positive controls induced the appropriate response.

This study is classified as **acceptable** and satisfies the Subdivision F Guideline requirement §84-2 for *in vitro* mutagenicity (mammalian forward gene mutation) data..

III. ISOPROPYLAMINE Salt of Dicamba

5. Ames Assay (MRID No.43310303)

CITATION:San, R., and D. Pugh (1994) *Salmonella* Plate Incorporation Mutagenicity Assay (Ames Test) with Confirmatory Assay. Microbiological Associates, Inc., Rockville, MD. Study # TE238.501014. 6/29/94. MRID 43310303. Unpublished.

EXECUTIVE SUMMARY: In a microbial mutagenicity assay (MRID 43310303), *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, or TA1538 were exposed to the IPA salt of dicamba (32.3% a.i.) in deionized distilled water at concentrations of 100, 333, 1000, 3333, or 5000 μg/plate in the presence and absence of mammalian metabolic activation. Preparations for metabolic activation were made from induced rat livers. The IPA salt of dicamba was tested up to the limit concentration of 5000 μg/plate and no cytotoxicity was observed. The positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background (reversion to prototrophy).**

This study is classified as **acceptable**, and satisfies the Subdivision F Guideline requirement §84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

6. *In Vitro* Mouse Lymphoma Assay (MRID No. 43310306)

CITATION: San, R., and J. Clarke. (1994) L5178Y/TK$^{+/-}$ Mouse lymphoma mutagenesis assay with a confirmatory assay. Microbiological Associates, Inc., Rockville, MD. Study # TE238.701020. 6/16/94. MRID No. 43310306. Unpublished.

EXECUTIVE SUMMARY: In a mammalian cell gene mutation assay at the thymidine kinase locus (MRID 43310306), L5178Y mouse lymphoma cells cultured *in vitro* were exposed to dicamba IPA salt (32.3% ai) in distilled water at concentrations of 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, and 5000 μg/mL in the presence and absence of S9 mammalian metabolic activation. Dicamba IPA salt was tested up to the limit dose. Under nonactivation conditions, the % total growth values over the evaluated dose range were from 92-101% (initial assay) and 51-107% (confirmatory assay). The mutation frequencies (MFs) for all of the treated cultures were $<2x$ the solvent controls. The S9-activation assay confirmed the findings of the nonactivation assay. The % total growth values were 75-126% (initial assay) and 49-114% (confirmatory assay). The MFs for all of the treated cultures were $<2x$ the solvent controls. Therefore, it was determined that **dicamba IPA salt was not mutagenic under either nonactivation or S9-activation conditions**. In both the nonactivated and activated conditions, the positive controls induced the appropriate response.

This study is classified as **acceptable** and satisfies the Subdivision F Guideline requirement §84-2 for *in vitro* mutagenicity (mammalian forward gene mutation) data.

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DATA EVALUATION REPORT

DIMETHYL AMINE SALT OF DICAMBA

Study Type: 84-2; *Salmonella typhimurium*/Mammalian Activation Gene Mutation Assay

Dynamac Study No. 115A (MRID 43310301)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticides Health Effects Group
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Date: 1/19/96

Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

[DICAMBA, DMA SALT] SALMONELLA/MAMMALIAN ACTIVATION; GENE MUTATION (84-2)

EPA Reviewer: Jess Rowland, M.S. Jess Rowland 7/2/96
Review Section II, Toxicology Branch II (7509C)

EPA Secondary Reviewer: Yiannakis Ioannou, Ph.D JM-I. 7/23/96
Review Section II, Toxicology Branch II (7509C)

DATA EVALUATION RECORD

STUDY TYPE: *Salmonella*/mammalian activation gene mutation assay

OPP Guideline Number: §84-2

DP BARCODE: D206005 SUBMISSION CODE: S470884

P.C. CODE: 029802 TOX. CHEM. NO.: **295B**

TEST MATERIAL (PURITY): Dimethylamine salt of dicamba (40.3% active ingredient)

SYNONYMS: DMA salt of dicamba, Banvel®

CITATION: San, R., and D. Pugh (1994) *Salmonella* Plate Incorporation Mutagenicity Assay (Ames Test) with Confirmatory Assay. Microbiological Associates, Inc., Rockville, MD. Study #. TE236.501014. June 29, 1994. **MRID No. 43310301.** Unpublished.

SPONSOR: Sandoz Agro, Inc., Des Plaines, IL

EXECUTIVE SUMMARY: In a microbial mutagenicity assay (MRID 43310301), *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, or TA1538 were exposed to the DMA salt of dicamba (40.3% a.i.) in deionized distilled water at concentrations of 100, 333, 1000, 3333, or 5000 μg/plate in the presence and absence of mammalian metabolic activation. Preparations for metabolic activation were made from induced rat livers.

The DMA salt of dicamba was tested up to the limit concentration of 5000 μg/plate and no cytotoxicity was observed. The positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background (reversion to prototrophy).**

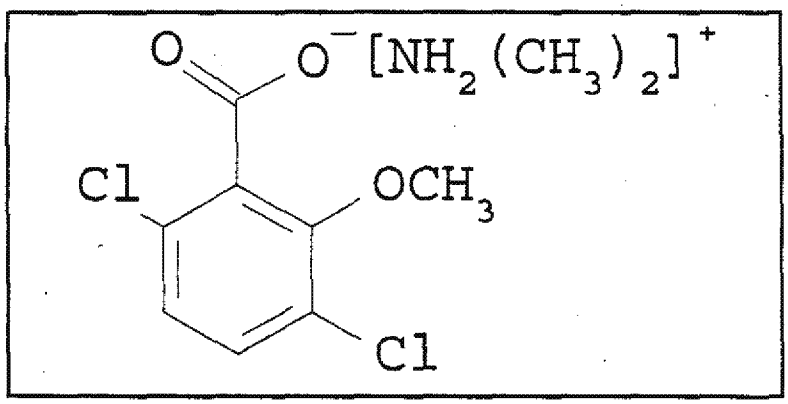
This study is classified as **acceptable**, and satisfies the requirements for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: DMA salt of dicamba

Description: Caramel color viscous liquid
Lot/Batch #: 5998-5
Purity: 40.3% a.i.
Stability of compound: Not reported
CAS No. 2300-66-5



Solvent used: Deionized distilled water (DDW)
Other comments: The test material was stored at room temperature and protected from light. Dosing solutions were prepared on the days of testing and aliquots of the low, mid, and high dose were analyzed by HPLC to confirm the nominal concentrations. The dosing solutions were 87-109% of the nominal concentrations.

2. Control Materials:

Negative: DDW
Solvent/final concentration: DDW/50 µL per plate
Positive: Nonactivation:

2-Nitrofluorene	1.0 µg/plate	TA98, TA1538
9-Aminoacridine	75.0 µg/plate	TA1537
Sodium azide	1.0 µg/plate	TA100, TA 1535

3. Activation: S9 derived from

X Aroclor 1254	X induced	X rat	X liver
__ phenobarbital	__ non-induced	__ mouse	__ lung
__ none		__ hamster	__ other
__ other			__ other

S9 mix composition: The S9 was prepared and stored frozen ($\leq$-70 C) until use. The S9 mix was prepared immediately prior to use and contained: S9 fraction (10% v/v), $MgCl_2$ (8 mM), KCl (33 mM), NADP (4 mM), glucose-6-phosphate (5 mM), and phosphate buffer (100 mM); 0.5 mL of S9 mix was used per culture flask.

4. Test organisms: S. typhimurium strains

__ TA97	X TA98	X TA100	__ TA102	__ TA104
X TA1535	X TA1537	X TA1538		

Properly maintained? Yes
Checked for appropriate genetic markers (rfa mutation, R factor)? Yes

5. Test compound concentrations used:

Preliminary cytotoxicity test: Ten dose levels (6.7, 10, 33, 67, 100, 333, 667, 1000, 3333, or 5000 μg/plate) were evaluated with strain TA100 in the presence and absence of S9 activation; single plates were used per dose, per condition; vehicle controls were included.

Mutagenicity assay: Five dose levels (100, 333, 1000, 3333, or 5000 μg/plate) were evaluated with strains TA98, TA100, TA1535, TA1537, or TA1538 in the presence and absence of S9 activation; triplicate plates were used for each dose, strain, and condition; vehicle and positive control groups were included. A confirmatory assay was also performed.

B. TEST PERFORMANCE

1. Type of Salmonella assay:
   - X standard plate test
   - __ pre-incubation (__ minutes)
   - __ "Prival" modification (i.e. azo-reduction method)
   - __ spot test
   - __ other

2. Protocol: Tester strains were inoculated into nutrient broth culture approximately 12 hours prior to dosing and incubated at 37 ± 2 C. Test substance and positive control substances were diluted in DDW to specified concentrations. Bacteria (100 µL), 50 µL of DDW, test substance, or positive control, and 0.5 mL of S9 mix were added to glass tubes containing 2 mL of melted top agar. The mixture was vortexed and poured on plates containing a layer of minimal agar medium. After the top agar solidified, the plates were inverted and incubated at 37 ± 2 C for approximately 48-72 hours. The plates were evaluated for gross toxic effects and total revertant colony numbers. Revertant colonies were counted either entirely by hand or by an automatic colony counter. The means and standard deviations for the mutation tests were determined from the counts of triplicate plates per strain, per dose, per condition.

3. Evaluation Criteria

   (a) Assay validity: The assay was considered acceptable if (1) the appropriate genetic markers were verified for each tester strain, (2) the number of spontaneous revertants for each tester strain was within specified limits, (3) the density of the tester strain cultures was $\geq 3x10^8$ cells/mL, and (4) the nonactivated and S9-activated positive controls induced at least a tripling of the number of revertants compared with the solvent controls.

   (b) Positive response: The test material was considered positive if it caused a dose-related increase in the mean number of revertants per plate of at least one strain. This increase must be at least 2-fold in strains TA98 and TA100 and at least 3-fold in strains TA1535, TA1537, and TA1538.

C. COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

II. RESULTS

A. Preliminary cytotoxicity assay: Ten doses of the test substance ranging from 6.7 to 5000 μg/plate were evaluated with and without S9 activation in single plate cultures using strain TA100. No compound precipitation or cytotoxicity was apparent at any of the nonactivated or S9-activated doses. Revertant colony counts were comparable to the vehicle controls.

B. Mutagenicity assay: Five doses of the test substance ranging from 100 to 5000 μg/plate were evaluated with and without S9 activation in triplicate plate cultures using strains TA98, TA100, TA1535, TA1537, or TA1538. The mutagenicity assays were performed in duplicate. Summary results from Tables 22 and 23 (study report pages 37-38) are appended to this DER. There were no significant differences in the number of revertant colonies in any tester strain at any dose level/condition in either the initial or repeat assays. Neither toxicity nor precipitate was observed in any tester strain with or without S9 activation in either assay. The positive control substances induced significant increases in revertant colonies in their respective strains. The vehicle controls responded in a similar manner to historical controls. Based on these results, the study authors concluded that the DMA salt of dicamba was not mutagenic in this microbial gene mutation assay.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. The reviewer agrees with the study authors' conclusions that the DMA salt of dicamba was assayed over an appropriate dose range and failed to induce a genotoxic response. Similarly, the sensitivity of the test system to detect mutagenesis was adequately demonstrated by the responses obtained with the nonactivated and S9-activated positive controls. This study is classified as acceptable.

B. Study deficiencies - None.

ATTACHMENTS

Salmonella Mutagenicity Assay
Summary of Results

Table 22

Test Article Id : DMA Salt of Dicamba
Study Number : TE236.501014 Experiment No : B1

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (μg)	TA98	TA100	TA1535	TA1537	TA1538
0.0	18 ± 5	159 ± 5	16 ± 3	7 ± 2	6 ± 5
100	24 ± 6	157 ± 10	11 ± 3	7 ± 2	9 ± 2
333	20 ± 2	152 ± 13	13 ± 6	6 ± 1	6 ± 4
1000	25 ± 2	157 ± 31	11 ± 2	4 ± 3	6 ± 1
3333	19 ± 4	152 ± 7	13 ± 5	5 ± 1	8 ± 1
5000	27 ± 1	150 ± 2	14 ± 5	5 ± 3	11 ± 2
Pos	333 ± 34	742 ± 40	1012 ± 558	691 ± 52	517 ± 84

Liver Microsomes: Rat liver S9

Dose (μg)	TA98	TA100	TA1535	TA1537	TA1538
0.0	34 ± 2	179 ± 9	16 ± 2	8 ± 3	15 ± 6
100	31 ± 8	169 ± 2	13 ± 4	6 ± 4	17 ± 4
333	27 ± 6	174 ± 4	15 ± 5	8 ± 3	16 ± 3
1000	30 ± 7	176 ± 26	15 ± 4	13 ± 6	15 ± 1
3333	31 ± 5	181 ± 14	16 ± 4	8 ± 4	19 ± 3
5000	27 ± 3	178 ± 20	11 ± 5	7 ± 3	15 ± 6
Pos	1085 ± 199	1384 ± 259	147 ± 33	123 ± 20	1536 ± 96

0.0 = Vehicle plating aliquot of 50 μl
Pos = Positive Control concentrations as specified in Materials and Methods section.

Salmonella Mutagenicity Assay
Summary of Results

Table 23

Test Article Id : DMA Salt of Dicamba
Study Number : TE236.501014 Experiment No : B2

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (μg)	TA98	TA100	TA1535	TA1537	TA1538
0.0	26 ± 3	189 ± 38	17 ± 3	7 ± 5	13 ± 3
100	27 ± 3	190 ± 12	20 ± 6	6 ± 2	9 ± 4
333	28 ± 7	173 ± 13	13 ± 4	7 ± 3	9 ± 2
1000	21 ± 1	180 ± 19	16 ± 5	13 ± 4	8 ± 1
3333	32 ± 3	179 ± 12	15 ± 2	10 ± 3	10 ± 1
5000	27 ± 4	196 ± 12	17 ± 4	8 ± 1	7 ± 2
Pos	286 ± 20	1255 ± 30	900 ± 35	760 ± 83	722 ± 17

Liver Microsomes: Rat liver S9

Dose (μg)	TA98	TA100	TA1535	TA1537	TA1538
0.0	40 ± 13	182 ± 11	18 ± 4	14 ± 6	20 ± 6
100	37 ± 8	181 ± 6	20 ± 4	14 ± 2	18 ± 7
333	41 ± 10	193 ± 3	16 ± 5	10 ± 2	19 ± 3
1000	33 ± 4	189 ± 4	20 ± 1	9 ± 3	19 ± 4
3333	37 ± 8	202 ± 23	21 ± 5	10 ± 5	26 ± 1
5000	43 ± 4	199 ± 20	22 ± 3	11 ± 3	21 ± 8
Pos	827 ± 128	973 ± 67	193 ± 105	66 ± 8	1356 ±1022

0.0 = Vehicle plating aliquot of 50 μl
Pos = Positive Control concentrations as specified in Materials and Methods section.

DATA EVALUATION REPORT

DIGLYCOLAMINE SALT OF DICAMBA

Study Type: 84-2; *Salmonella typhimurium*/Mammalian Activation Gene Mutation Assay

Dynamac Study No. 115B (MRID 43310302)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
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Prepared by

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Sciences Division
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Reto Engler, Ph.D.
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Date: 1/19/96

Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

[DICAMBA, DGA SALT] SALMONELLA/MAMMALIAN ACTIVATION; GENE MUTATION (84-2)

EPA Reviewer: Jess Rowland, M.S. 7/2/96
Review Section II, Toxicology Branch II (7509C)

EPA Secondary Reviewer: Yiannakis Ioannou, Ph.D 7/23/96
Review Section II, Toxicology Branch II (7509C)

DATA EVALUATION RECORD

STUDY TYPE: *Salmonella*/mammalian activation gene mutation assay

OPP Guideline Number: §84-2

DP BARCODE: D206005 SUBMISSION CODE: S470884

P.C. CODE: 128931 TOX. CHEM. NO.: **295F**

TEST MATERIAL (PURITY): Diglycolamine salt of dicamba (39.7% active ingredient)

SYNONYMS: DGA salt of dicamba

CITATION: San, R., and D. Pugh (1994) *Salmonella* Plate Incorporation Mutagenicity Assay (Ames Test) with Confirmatory Assay. Microbiological Associates, Inc., Rockville, MD. Study #. TE237.501014. June 29, 1994. **MRID No.43310302.** Unpublished.

SPONSOR: Sandoz Agro, Inc., Des Plaines, IL

EXECUTIVE SUMMARY: In a microbial mutagenicity assay (MRID 43310302), *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, or TA1538 were exposed to the DGA salt of dicamba (39.7% a.i.) in deionized distilled water at concentrations of 100, 333, 1000, 3333, or 5000 μg/plate in the presence and absence of mammalian metabolic activation. Preparations for metabolic activation were made from induced rat livers.

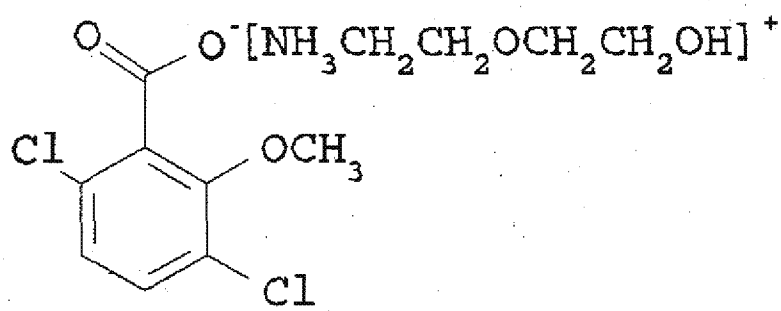
The DGA salt of dicamba was tested up to the limit concentration of 5000 μg/plate, but no cytotoxicity was observed. The positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background (reversion to prototrophy).**

This study is classified as **acceptable**, and satisfies the requirements for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: DGA salt of dicamba
   Description: Caramel color viscous liquid
   Lot/Batch #: 5998-1
   Purity: 39.7% a.i.
   Stability of compound: Not reported
   CAS No. 104040-79-1



Solvent used: Deionized distilled water (DDW)
Other comments: The test material was stored at room temperature and protected from light. Dosing solutions were prepared on the days of testing and aliquots of the low, mid, and high dose were analyzed by HPLC to confirm the nominal concentrations. The dosing solutions were 87-106% of the nominal concentrations.

2. Control Materials:

Negative: DDW
Solvent/final concentration: DDW/50 μL per plate
Positive: Nonactivation:
2-Nitrofluorene 1.0 μg/plate TA98, TA1538
9-Aminoacridine 75.0 μg/plate TA1537
Sodium azide 1.0 μg/plate TA100, TA 1535

Activation:
2-Aminoanthracene (2-anthramine) 1.0 μg/plate all strains

3. Activation: S9 derived from

X Aroclor 1254	X induced	X rat	X liver
__ phenobarbital	__ non-induced	__ mouse	__ lung
__ none		__ hamster	__ other
__ other			__ other

S9 mix composition: The S9 was prepared and stored frozen (≤-70 C) until use. The S9 mix was prepared immediately prior to use and contained: S9 fraction (10% v/v), $MgCl_2$ (8 mM), KCl (33 mM), NADP (4 mM), glucose-6-phosphate (5 mM), and phosphate buffer (100 mM); 0.5 mL of S9 mix was used per culture flask.

4. Test organisms: S. typhimurium strains

__ TA97	X TA98	X TA100	__ TA102	__ TA104
X TA1535	X TA1537	X TA1538		

Properly maintained? Yes
Checked for appropriate genetic markers (rfa mutation, R factor)? Yes

5. Test compound concentrations used:

Preliminary cytotoxicity test: Ten dose levels (6.7, 10, 33, 67, 100, 333, 667, 1000, 3333, or 5000 μg/plate) were evaluated with strain TA100 in the presence and absence of S9 activation; single plates were used per dose, per condition; vehicle controls were included.

Mutagenicity assay: Five dose levels (100, 333, 1000, 3333, or 5000 μg/plate) were evaluated with strains TA98, TA100, TA1535, TA1537, or TA1538 in the presence and absence of S9 activation; triplicate plates were used for each dose, strain, and condition; vehicle and positive control groups were included. A confirmatory assay was also performed.

B. TEST PERFORMANCE

1. Type of Salmonella assay:
   - X standard plate test
   - __ pre-incubation (__ minutes)
   - __ "Prival" modification (i.e. azo-reduction method)
   - __ spot test
   - __ other

2. Protocol: Tester strains were inoculated into nutrient both culture approximately 12 hours prior to dosing and incubated at 37 ± 2 C. Test substance and positive control substances were diluted in DDW to specified concentrations. Bacteria (100 μL), 50 μL of DDW, test substance, or positive control, and 0.5 mL of S9 mix were added to glass tubes containing 2 mL of melted top agar. The mixture was vortexed and poured on plates containing a layer of minimal agar medium. After the top agar solidified, the plates were inverted and incubated at 37 ± 2 C for approximately 48-72 hours. The plates were evaluated for gross toxic effects and total revertant colony numbers. Revertant colonies were counted either entirely by hand or by an automatic colony counter. The means and standard deviations for the mutation tests were determined from the counts of triplicate plates per strain, per dose, per condition.

3. Evaluation Criteria

   (a) Assay validity: The assay was considered acceptable if (1) the appropriate genetic markers were verified for each tester strain, (2) the number of spontaneous revertants for each tester strain was within specified limits, (3) the density of the tester strain cultures was $\geq 3 \times 10^8$ cells/mL, and (4) the nonactivated and S9-activated positive controls induced at least a tripling of the number of revertants compared with the solvent controls.

   (b) Positive response: The test material was considered positive if it caused a dose-related increase in the mean number of revertants per plate of at least one strain. This increase must be at least 2-fold in strains TA98 and TA100 and at least 3-fold in strains TA1535, TA1537, and TA1538.

C. COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality were provided.

II. REPORTED RESULTS

A. Preliminary cytotoxicity assay: Ten doses of the test substance ranging from 6.7 to 5000 μg/plate were evaluated with and without S9 activation in single plate cultures using strain TA100. No compound precipitation or cytotoxicity was apparent at any of the nonactivated or S9-activated doses. Revertant colony counts were comparable to the vehicle controls.

B. Mutagenicity assay: Five doses of the test substance ranging from 100 to 5000 μg/plate were evaluated with and without S9 activation in triplicate plate cultures using strains TA98, TA100, TA1535, TA1537, or TA1538. The mutagenicity assays were performed in duplicate. Summary results from Tables 22 and 23 (study report pages 37-38) are appended to this DER. There were no significant differences in the number of revertant colonies in any tester strain at any dose level/condition in either the initial or repeat assays. Neither toxicity nor precipitate was observed in any tester strain with or without S9 activation in either assay. The positive control substances induced significant increases in revertant colonies in their respective strains. The vehicle controls responded in a similar manner to historical controls. Based on these results, the study authors concluded that the DGA salt of dicamba was not mutagenic in this microbial gene mutation assay.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. The reviewer agrees with the study authors' conclusions that the DGA salt of dicamba was assayed over an appropriate dose range and failed to induce a genotoxic response. Similarly, the sensitivity of the test system to detect mutagenesis was adequately demonstrated by the responses obtained with the nonactivated and S9-activated positive controls. This study is classified as acceptable.

B. Study deficiencies - None.

ATTACHMENTS

Salmonella Mutagenicity Assay
Summary of Results

Table 22

Test Article Id : DGA Salt of Dicamba
Study Number : TE237.501014 Experiment No : B1

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (μg)	TA98	TA100	TA1535	TA1537	TA1538
0.0	17 ± 4	151 ± 4	13 ± 3	6 ± 3	9 ± 1
100	15 ± 3	145 ± 16	12 ± 1	4 ± 2	10 ± 2
333	19 ± 3	142 ± 18	9 ± 4	7 ± 3	7 ± 1
1000	15 ± 6	151 ± 9	12 ± 4	6 ± 2	7 ± 3
3333	25 ± 6	159 ± 10	10 ± 2	5 ± 2	4 ± 2
5000	17 ± 4	157 ± 7	10 ± 4	6 ± 1	8 ± 2
Pos	276 ± 21	748 ± 59	542 ± 6	409 ± 73	470 ± 22

Liver Microsomes: Rat liver S9

Dose (μg)	TA98	TA100	TA1535	TA1537	TA1538
0.0	30 ± 1	159 ± 13	12 ± 1	6 ± 2	18 ± 1
100	27 ± 6	161 ± 25	12 ± 6	6 ± 1	15 ± 3
333	32 ± 4	166 ± 12	15 ± 6	7 ± 3	14 ± 4
1000	33 ± 9	153 ± 10	16 ± 6	8 ± 5	12 ± 4
3333	26 ± 4	166 ± 21	11 ± 6	7 ± 1	10 ± 4
5000	29 ± 3	128 ± 48	10 ± 3	6 ± 1	11 ± 3
Pos	822 ± 105	901 ± 58	117 ± 22	85 ± 4	822 ± 50

0.0 = Vehicle plating aliquot of 50 μl
Pos = Positive Control concentrations as specified in Materials and Methods section.

Salmonella Mutagenicity Assay
Summary of Results

Table 23

Test Article Id : DGA Salt of Dicamba
Study Number : TE237.501014 Experiment No : B2

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (μg)	TA98	TA100	TA1535	TA1537	TA1538
0.0	21 ± 9	162 ± 11	18 ± 2	9 ± 4	12 ± 3
100	21 ± 2	167 ± 12	19 ± 2	6 ± 1	10 ± 2
333	23 ± 7	153 ± 6	11 ± 1	8 ± 2	9 ± 4
1000	25 ± 3	162 ± 16	12 ± 5	5 ± 2	11 ± 3
3333	24 ± 2	188 ± 3	13 ± 7	8 ± 6	9 ± 1
5000	34 ± 2	177 ± 16	15 ± 5	7 ± 1	14 ± 6
Pos	270 ± 25	989 ± 38	783 ± 59	529 ± 151	481 ± 66

Liver Microsomes: Rat liver S9

Dose (μg)	TA98	TA100	TA1535	TA1537	TA1538
0.0	41 ± 2	174 ± 27	13 ± 5	5 ± 2	15 ± 1
100	33 ± 10	182 ± 19	17 ± 5	8 ± 1	12 ± 7
333	32 ± 2	181 ± 6	17 ± 3	9 ± 2	14 ± 3
1000	34 ± 2	177 ± 9	18 ± 5	8 ± 2	17 ± 3
3333	39 ± 3	175 ± 10	15 ± 4	9 ± 5	18 ± 3
5000	35 ± 5	173 ± 17	17 ± 6	9 ± 5	7 ± 3
Pos	1596 ± 246	1741 ± 169	171 ± 14	150 ± 26	1875 ± 17

0.0 = Vehicle plating aliquot of 50 μl
Pos = Positive Control concentrations as specified in Materials and Methods section.

012000

DATA EVALUATION RECORD

AMINE SALTS OF DICAMBA-DMA (Dimethylamine Salt)

Study Type: 84-2; Mammalian Cells in Culture - Gene Mutation Assay in Mouse Lymphoma Cells

Work Assignment No. 1-15D (MRID 43310304)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticides Health Effects Group
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Date: 4/15/96

Project Manager:
William Spangler, Ph.D.

Signature: _____
Date: 4/10/96

Quality Assurance:
Reto Engler, Ph.D.

Signature: _____
Date: 4/10/96

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Jess Rowland, M.S Jess Rowland 7/24/96
Review Section II, Toxicology Branch II

EPA Secondary Reviewer: Yiannakis Ioannou, Ph.D, Y.M.I 7/23/96
Review Section II, Toxicology Branch II

DATA EVALUATION RECORD

STUDY TYPE: Mammalian cells in culture - gene mutation assay - mouse lymphoma cells

OPP Guideline Number: §84-2

DP BARCODE: D206005 SUBMISSION CODE: S470884

P.C. CODE: 029802 TOX. CHEM. NO.: **295B**

TEST MATERIAL (PURITY): Dicamba DMA salt (40.3% ai)

SYNONYMS: Dimethylamine salt of 3,6-dichloro-*o*-anisic acid

CITATION: San, R., and J. Clarke. (1994) L5178Y/TK$^{+/-}$ Mouse lymphoma mutagenesis assay with a confirmatory assay. Microbiological Associates, Inc., Rockville, MD. Study # TE236.701020. June 21, 1994. **MRID No. 43310304.** Unpublished.

SPONSOR: Sandoz Agro, Inc., Des Plaines, IL

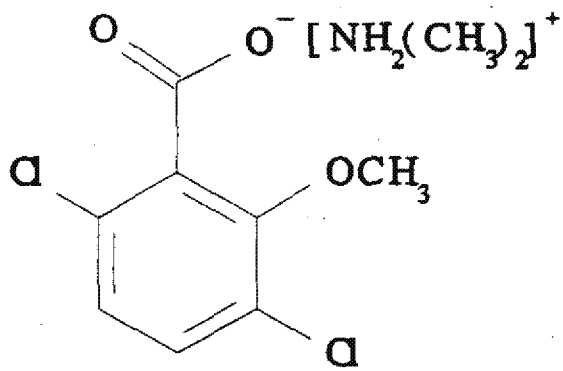
EXECUTIVE SUMMARY: In a mammalian cell gene mutation assay at the thymidine kinase locus (MRID 43310304), L5178Y mouse lymphoma cells cultured *in vitro* were exposed to dicamba DMA salt (40.3% ai) in distilled water at concentrations of 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, and 5000 μg/mL in the presence and absence of S9 mammalian metabolic activation. Dicamba DMA salt was tested up to the limit dose. Under nonactivation conditions, the % total growth values over the evaluated dose range were from 69-109% (initial assay) and 65-111% (confirmatory assay). The mutation frequencies (MFs) for all of the treated cultures were <2x the solvent controls; the exception was the 4500 μg/mL dose, which had a MF of approximately 2x background in the confirmatory trial. However, the 4500 μg/mL response was not reproducible. The S9-activation assay confirmed the findings of the nonactivation assay. The % total growth values were 26-109% (initial assay) and 23-113% (confirmatory assay). The MFs for all of the treated cultures were <2x the solvent controls with the exception of the 3000 μg/mL dose in the confirmatory trial which had a MF of approximately 2x background; this result was not reproducible. It was determined that **dicamba DMA salt was not mutagenic under either nonactivation or S9-activation conditions.** In both the nonactivated and activated conditions, the positive controls induced the appropriate response.

This study is classified as **acceptable,** satisfies the guideline requirements for **in vitro** mutagenicity (mammalian forward gene mutation) data (§84-2).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Dicamba DMA salt
   Description: Caramel-colored viscous liquid
   Lot/Batch #: 5998-5
   Purity: 40.3% ai
   Stability of compound: Not provided
   CAS #: 2300-66-5
   Structure:



   Solvent used: Sterile distilled water
   Other comments: The test material was stored at room temperature, protected from light. Dosing solutions were prepared under amber lights and kept in darkness throughout the 4-hour exposure period. Actual concentrations of the low, mid, and high doses used in the initial and confirmatory mutagenicity assays were verified analytically by HPLC. The dosing solutions were from 92.8-110% of the nominal concentrations for the two replicate trials (study report page 26).

2. Control Materials
   Negative: The negative control was the test material solvent, water.
   Solvent/final concentration: Sterile distilled water (1%, v/v)
   Positive:
   Nonactivation: Ethyl methanesulfonate was used at concentrations of 0.25 and 0.5 μL/mL with DMSO as the solvent.
   Activation: 7,12-Dimethylbenz(a)anthracene was used at concentrations of 2.5 and 5.0 μg/mL. The solvent was DMSO.

3. Activation

S-9 was derived from:

X	Aroclor 1254	X	Induced	X	Rat	X	Liver
	Phenobarbital		Non-induced		Mouse		Lung
	None				Hamster		Other
	Other				Other		

The S9 mix was prepared by the testing laboratory and contained DL-isocitric acid (11.25 mg), NADP (6.0 mg), Fischers's medium with 0.1% pluronics (0.75 mL), and S9 homogenate (0.25 mL).

4. Test Cells

Mouse lymphoma L5178Y cells were used in the study.

Properly maintained? **Yes**
Periodically checked for mycoplasma contamination? **Not reported**
Periodically checked for karyotype stability? **Not reported**
Periodically "cleansed" against high spontaneous background? **Yes**

Media: Fischer's Medium for Leukemic Cells of Mice with 0.1% pluronic solution supplemented with heat-inactivated horse serum (10%, v:v) and 4mM L-glutamine

5. Locus Examined

Thymidine kinase (TK)
Selection agent: 3 µg/mL trifluorothymidine (TFT)

6. Test compound concentrations used

a. Preliminary Assays:

Nonactivated and activated conditions: Nine doses (0.5, 1.0, 5.0, 10, 50, 100, 500, 1000, and 5000 µg/mL) were tested with and without S9 activation. The suspension growth (Day 1 cell concentration/0.3×10^6 cells/mL) x (Day 2 cell concentration/Day 1 adjusted cell concentration) were determined as a measure of toxicity for all evaluated levels.

b. Mutation Assays:

Nonactivated and activated conditions: Ten doses (900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, and 5000 µg/mL) were tested with and without S9 activation. MFs were determined for all evaluated levels. Initial and confirmatory trials were assayed identically.

B. TEST PERFORMANCE

1. Cell treatment

a. Cells exposed to test compound, negative/solvent or positive controls for:
4 hours (nonactivated); 4 hours (activated)

b. After washing, cells were cultured for 2 days (expression period) before cell selection.

c. After expression, 1 x 10^6 cells/dish (3 dishes/group) were cultured for 10-12 days in selection medium to determine numbers of mutants, and 200 cells/dish (3 dishes/group) were cultured for 10-12 days without selective agent to determine cloning efficiency.

2. Statistical Methods

The data were not evaluated for statistical significance.

3. Evaluation Criteria

a. Assay validity: The assay was considered valid if (i) the mutant frequencies (MFs) for the positive controls is ≥2x the solvent controls, (ii) the spontaneous MFs (for the solvent controls) must be from 20 to 100 per 10^6 surviving cells, (iii) the cloning efficiency of the solvent controls must be >50%.

b. Positive result: The test material was considered mutagenic if it caused a dose-related increase in the MFs with at least two dose levels in the ≥10% total growth range demonstrating MFs that are 2-fold greater than background. Alternatively, the test material was considered mutagenic if it caused a reproducible 2-fold increase for at least one dose level.

C. COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

II. REPORTED RESULTS

A. Preliminary cytotoxicity assay

Nine preliminary doses of the dicamba DMA salt (0.5, 1.0, 5.0, 10, 50, 100, 500, 1000, and 5000 µg/mL) were evaluated with and without S9 activation. Suspension growths were determined as a measure of toxicity for all evaluated levels. The test material was soluble at all dose levels. At the 5000 µg/mL dose level, the suspension growth was depressed by 3% (-S9) and by 15% (+S9) relative to the solvent controls. Based on these results, the mutation assays were conducted with ten doses (900-5000 µg/mL) with and without S9 activation.

B. Mutagenicity assay

The test material was soluble at all dose levels evaluated with and without S9 activation (900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, and 5000 µg/mL). Initial and confirmatory trials were assayed at these dose levels. Under both the activation and nonactivation conditions for the initial and confirmatory trials, all of the following criteria for assay validity were met: (i) the mutant frequencies (MFs) for the positive controls were ≥2x the solvent controls, (ii) the spontaneous MFs (for the solvent controls) were from 20 to 100 per 10^6 cells, and (iii) the cloning efficiency of the solvent controls were >50%.

Each trial included three cultures per dose level, four solvent controls (water) for the test article, two solvent controls (DMSO) for the positive controls, and two concentrations of the positive control. Results from the initial and confirmatory trials of the mutation assay are presented in Appendices 1-4 (Tables 2, 4, 6, and 8; study report pages 15, 17, 19, and 21, respectively) included in this DER and summarized as follows:

Nonactivation conditions: The % total growth values [(% suspension growth x % cloning growth)/100] over the evaluated dose range were from 69-109% for the initial assay and 65-111% for the confirmatory assay. The MFs for the treated cultures were 18-39 per 10^6 cells for the initial assay (Table 2, study report page 15) and 32-69 per 10^6 cells for the confirmatory assay (Table 6, study report page 19). The average MFs for the solvent controls were 22 and 34 per 10^6 cells for the initial and confirmatory assays, respectively. A 2-fold increase in the MFs was reported for the 4500 µg/mL dose (69 vs. 34 per 10^6 cells for the background, confirmatory trial); however, it did not occur in the initial trial. All other MFs for cultures treated with dicamba DMA salt were <2x the background. Therefore, it was determined that the dicamba DMA salt was not mutagenic under the nonactivation conditions.

S9-activation conditions: The % total growth values over the evaluated dose range were from 26-109% for the initial assay and 23-113% for the confirmatory assay. The MFs for the treated cultures were 37-49 per 10^6 cells for the initial assay (Table 4, study report page 17) and 58-117 per 10^6 cells for the confirmatory assay (Table 8, study report page 21). The average MFs for the solvent controls were 30 and 56 per 10^6 cells for the initial and confirmatory assays, respectively. A 2-fold increase in the MFs was reported for the 3000 µg/mL dose (117 vs. 56 per 10^6 cells for the background, confirmatory trial); however, it did not occur in the initial trial. All other MFs for the treated cultures were <2x the background. Therefore, it was determined that dicamba DMA salt was not a mutagen under the S9-activation conditions.

III. DISCUSSION/CONCLUSIONS

A. Investigator's Conclusions

The study authors concluded that, under the conditions of this study, dicamba DMA salt was not mutagenic in the presence or absence of S9 mammalian metabolic activation.

B. Reviewer's Discussion

We agree with the study authors' conclusion that dicamba DMA salt was not mutagenic under the conditions of this study. The dose levels tested were adequate for both the nonactivation and activation conditions as it was tested to the limit dose. The positive controls induced MFs that were >2x the background. Under both the S9 activated and the nonactivation conditions, one dose level in the confirmatory trials induced MFs approximately 2x the solvent controls. However, because it was not reproducible and all other MFs for the treated cultures were <2x the background, dicamba DMA salt was not considered mutagenic in this assay.

IV. STUDY DEFICIENCIES

None.

APPENDIX 1

TABLE 2

CLONING DATA FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS
TREATED WITH DMA Salt of Dicamba
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION
INITIAL ASSAY

Test Article Concentration (µg/ml)	Ave #/ TFT Plate[a]	TFT Stand Dev	Ave #/ V.C. Plate[a]	V.C. Stand Dev	Mutant Frequency[b]	Induced Mutant Frequency[c]	% Total Growth[d]
5000	28/3	2	149/3	14	38	16	72
4500	27/3	3	140/3	5	39	17	69
4000	20/3	3	131/3	10	31	9	75
3500	16/3	4	149/3	9	21	-1	89
3000	12/3	4	130/3	7	18	-4	84
2500	24/3	3	149/3	5	32	10	102
2000	25/3	5	136/3	16	37	15	94
1500	16/2	3	156/3	12	21	-1	104
1000	18/3	3	157/3	11	23	1	109
900	20/3	5	131/3	18	31	9	98
Solvent 1	15/3	3	163/3	15	18		
Solvent 2	15/3	5	157/3	14	19		
Solvent 3	10/3	3	121/3	17	17		
Solvent 4	21/3	7	131/3	5	32		
Mean Solvent Mutant Frequency= 22							
Positive Control - Ethyl Methanesulfonate (µl/ml)							
0.50	359/3	6	83/3	5	865	839	30
0.25	241/3	9	134/3	10	360	334	69
Solvent 1	18/3	1	144/3	12	25		
Solvent 2	19/3	1	141/3	14	27		
Mean Solvent Mutant Frequency= 26							

[a] - Average # of colonies per plate and # of plates scored

[b] - Mutant frequency (per 10^6 surviving cells) = (Average # TFT colonies / average # VC colonies) x 200

[c] - Induced mutant frequency (per 10^6 surviving cells) = mutant frequency - average mutant frequency of solvent controls

[d] - % total growth = (% suspension growth x % cloning growth) / 100

APPENDIX 2

TABLE 4

CLONING DATA FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS
TREATED WITH DMA Salt of Dicamba
IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION
INITIAL ASSAY

Test Article Concentration (µg/ml)	Ave #/ TFT Plate[a]	TFT Stand Dev	Ave #/ V.C. Plate[a]	V.C. Stand Dev	Mutant Frequency[b]	Induced Mutant Frequency[c]	% Total Growth[d]
5000	34/3	7	155/3	15	44	14	26
4500	35/3	9	+				
4000	29/2	8	129/3	15	45	15	52
3500	34/3	2	148/3	19	46	16	71
3000	33/3	1	136/3	3	49	19	76
2500	28/3	2	131/3	9	43	13	83
2000	28/3	5	152/3	19	37	7	92
1500	27/3	3	146/3	9	37	7	98
1000	32/3	2	159/3	20	40	10	108
900	35/3	5	162/3	17	43	13	109
Solvent 1	27/3	4	138/3	1	39		
Solvent 2	15/3	3	144/3	13	21		
Solvent 3	22/3	3	141/3	8	31		
Solvent 4	21/3	3	142/3	14	30		
Mean Solvent Mutant Frequency= 30							
Positive Control - 7,12 Dimethylbenz(a)anthracene (µg/ml)							
5.0	142/3	4	47/3	7	604	565	11
2.5	109/3	17	127/3	12	172	133	75
Solvent 1	29/3	6	127/3	10	46		
Solvent 2	20/3	2	129/3	12	31		
Mean Solvent Mutant Frequency= 39							

+ - Culture lost

[a] - Average # of colonies per plate and # of plates scored

[b] - Mutant frequency (per 10^6 surviving cells) = (Average # TFT colonies / average # VC colonies) x 200

[c] - Induced mutant frequency (per 10^6 surviving cells) = mutant frequency - average mutant frequency of solvent controls

[d] - % total growth = (% suspension growth x % cloning growth) / 100

APPENDIX 3

TABLE 6

CLONING DATA FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS
TREATED WITH DNA Salt of Dicamba
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION
CONFIRMATORY ASSAY

Test Article Concentration (µg/ml)	Ave #/ TFT Plate[a]	TFT Stand Dev	Ave #/ V.C. Plate[a]	V.C. Stand Dev	Mutant Frequency[b]	Induced Mutant Frequency[c]	% Total Growth[d]
5000	42/3	5	144/3	5	58	24	65
4500	50/3	14	145/3	18	69	35	75
4000	34/3	1	151/3	15	45	11	85
3500	31/3	7	130/3	10	48	14	75
3000	37/3	6	141/3	9	52	18	84
2500	33/3	1	133/3	13	50	16	83
2000	25/3	1	156/3	21	32	-2	104
1500	35/3	5	142/3	2	49	15	102
1000	27/3	4	151/3	14	36	2	111
900	36/3	2	137/3	22	53	19	97
Solvent 1	18/3	5	130/3	6	28		
Solvent 2	23/3	3	140/3	17	33		
Solvent 3	27/3	5	149/3	10	36		
Solvent 4	26/3	9	138/3	7	38		
Mean Solvent Mutant Frequency= 34							
Positive Control - Ethyl Methanesulfonate (µl/ml)							
0.50	328/3	24	74/3	2	886	854	34
0.25	293/3	24	103/3	8	569	537	60
Solvent 1	19/3	1	143/3	5	27		
Solvent 2	24/3	6	132/3	7	36		
Mean Solvent Mutant Frequency= 32							

[a] - Average # of colonies per plate and # of plates scored

[b] - Mutant frequency (per 10^6 surviving cells) = (Average # TFT colonies / average # VC colonies) x 200

[c] - Induced mutant frequency (per 10^6 surviving cells) = mutant frequency - average mutant frequency of solvent controls

[d] - % total growth = (% suspension growth x % cloning growth) / 100

APPENDIX 4

TABLE 8

CLONING DATA FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS
TREATED WITH DNA Salt of Dicamba
IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION
CONFIRMATORY ASSAY

Test Article Concentration (µg/ml)	Ave #/ TFT Plate[a]	TFT Stand Dev	Ave #/ V.C. Plate[a]	V.C. Stand Dev	Mutant Frequency[b]	Induced Mutant Frequency[c]	% Total Growth[d]
5000	41/3	5	93/3	8	88	32	23
4500	49/3	6	107/3	10	92	36	30
4000	41/3	1	123/3	10	67	11	44
3500	50/3	3	117/3	9	85	29	49
3000	62/3	3	106/3	8	117	61	56
2500	55/3	3	135/3	9	81	25	81
2000	65/3	1	139/3	23	94	38	88
1500	54/3	5	139/3	22	78	22	95
1000	41/3	3	130/3	6	63	7	94
900	43/3	1	148/3	16	58	2	113
Solvent 1	40/3	5	128/3	9	63		
Solvent 2	43/3	7	131/3	23	66		
Solvent 3	31/3	5	120/3	9	52		
Solvent 4	29/3	11	133/3	4	44		
Mean Solvent Mutant Frequency= 56							
Positive Control - 7,12 Dimethylbenz(a)anthracene (µg/ml)							
5.0	144/3	11	82/3	13	351	293	43
2.5	118/3	10	118/3	5	200	142	77
Solvent 1	37/3	1	124/3	1	60		
Solvent 2	37/3	4	135/3	5	55		
Mean Solvent Mutant Frequency= 58							

[a] - Average # of colonies per plate and # of plates scored

[b] - Mutant frequency (per 10^6 surviving cells) = (Average # TFT colonies / average # VC colonies) x 200

[c] - Induced mutant frequency (per 10^6 surviving cells) = mutant frequency - average mutant frequency of solvent controls

[d] - % total growth = (% suspension growth x % cloning growth) / 100

DATA EVALUATION REPORT

DIGLYCOLAMINE SALT OF DICAMBA

Study Type: 84-2; *Salmonella typhimurium*/Mammalian Activation Gene Mutation Assay

Dynamac Study No. 115B (MRID 43310302)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticides Health Effects Group
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Reto Engler, Ph.D.

Signature: Reto Engler by MF
Date: 1/19/96

Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

[DICAMBA, DGA SALT] SALMONELLA/MAMMALIAN ACTIVATION; GENE MUTATION (84-2)

EPA Reviewer: Jess Rowland, M.S. 7/2/96
Review Section II, Toxicology Branch II (7509C)

EPA Secondary Reviewer: Yiannakis Ioannou, Ph.D 7/23/96
Review Section II, Toxicology Branch II (7509C)

DATA EVALUATION RECORD

STUDY TYPE: *Salmonella*/mammalian activation gene mutation assay

OPP Guideline Number: §84-2

DP BARCODE: D206005 SUBMISSION CODE: S470884

P.C. CODE: 128931 TOX. CHEM. NO.: **295F**

TEST MATERIAL (PURITY): Diglycolamine salt of dicamba (39.7% active ingredient)

SYNONYMS: DGA salt of dicamba

CITATION: San, R., and D. Pugh (1994) *Salmonella* Plate Incorporation Mutagenicity Assay (Ames Test) with Confirmatory Assay. Microbiological Associates, Inc., Rockville, MD. Study #. TE237.501014. June 29, 1994. **MRID No.43310302.** Unpublished.

SPONSOR: Sandoz Agro, Inc., Des Plaines, IL

EXECUTIVE SUMMARY: In a microbial mutagenicity assay (MRID 43310302), *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, or TA1538 were exposed to the DGA salt of dicamba (39.7% a.i.) in deionized distilled water at concentrations of 100, 333, 1000, 3333, or 5000 μg/plate in the presence and absence of mammalian metabolic activation. Preparations for metabolic activation were made from induced rat livers.

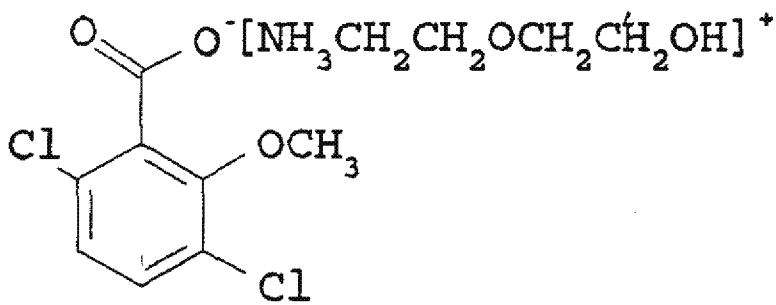
The DGA salt of dicamba was tested up to the limit concentration of 5000 μg/plate, but no cytotoxicity was observed. The positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background (reversion to prototrophy).**

This study is classified as **acceptable**, and satisfies the requirements for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: DGA salt of dicamba
   Description: Caramel color viscous liquid
   Lot/Batch #: 5998-1
   Purity: 39.7% a.i.
   Stability of compound: Not reported
   CAS No. 104040-79-1



   Solvent used: Deionized distilled water (DDW)
   Other comments: The test material was stored at room temperature and protected from light. Dosing solutions were prepared on the days of testing and aliquots of the low, mid, and high dose were analyzed by HPLC to confirm the nominal concentrations. The dosing solutions were 87-106% of the nominal concentrations.

2. Control Materials:

   Negative: DDW
   Solvent/final concentration: DDW/50 µL per plate
   Positive: Nonactivation:
   2-Nitrofluorene 1.0 µg/plate TA98, TA1538
   9-Aminoacridine 75.0 µg/plate TA1537
   Sodium azide 1.0 µg/plate TA100, TA 1535

   Activation:
   2-Aminoanthracene (2-anthramine) 1.0 µg/plate all strains

3. Activation: S9 derived from

X Aroclor 1254	X induced	X rat	X liver
__ phenobarbital	__ non-induced	__ mouse	__ lung
__ none		__ hamster	__ other
__ other			__ other

S9 mix composition: The S9 was prepared and stored frozen (≤-70 C) until use. The S9 mix was prepared immediately prior to use and contained: S9 fraction (10% v/v), $MgCl_2$ (8 mM), KCl (33 mM), NADP (4 mM), glucose-6-phosphate (5 mM), and phosphate buffer (100 mM); 0.5 mL of S9 mix was used per culture flask.

4. Test organisms: S. typhimurium strains

__ TA97	X TA98	X TA100	__ TA102	__ TA104
X TA1535	X TA1537	X TA1538		

Properly maintained? Yes
Checked for appropriate genetic markers (rfa mutation, R factor)? Yes

5. Test compound concentrations used:

Preliminary cytotoxicity test: Ten dose levels (6.7, 10, 33, 67, 100, 333, 667, 1000, 3333, or 5000 μg/plate) were evaluated with strain TA100 in the presence and absence of S9 activation; single plates were used per dose, per condition; vehicle controls were included.

Mutagenicity assay: Five dose levels (100, 333, 1000, 3333, or 5000 μg/plate) were evaluated with strains TA98, TA100, TA1535, TA1537, or TA1538 in the presence and absence of S9 activation; triplicate plates were used for each dose, strain, and condition; vehicle and positive control groups were included. A confirmatory assay was also performed.

B. TEST PERFORMANCE

1. Type of Salmonella assay:
   - X standard plate test
   - __ pre-incubation (__ minutes)
   - __ "Prival" modification (i.e. azo-reduction method)
   - __ spot test
   - __ other

2. Protocol: Tester strains were inoculated into nutrient both culture approximately 12 hours prior to dosing and incubated at 37 ± 2 C. Test substance and positive control substances were diluted in DDW to specified concentrations. Bacteria (100 μL), 50 μL of DDW, test substance, or positive control, and 0.5 mL of S9 mix were added to glass tubes containing 2 mL of melted top agar. The mixture was vortexed and poured on plates containing a layer of minimal agar medium. After the top agar solidified, the plates were inverted and incubated at 37 ± 2 C for approximately 48-72 hours. The plates were evaluated for gross toxic effects and total revertant colony numbers. Revertant colonies were counted either entirely by hand or by an automatic colony counter. The means and standard deviations for the mutation tests were determined from the counts of triplicate plates per strain, per dose, per condition.

3. Evaluation Criteria

   (a) Assay validity: The assay was considered acceptable if (1) the appropriate genetic markers were verified for each tester strain, (2) the number of spontaneous revertants for each tester strain was within specified limits, (3) the density of the tester strain cultures was $\geq 3 \times 10^8$ cells/mL, and (4) the nonactivated and S9-activated positive controls induced at least a tripling of the number of revertants compared with the solvent controls.

   (b) Positive response: The test material was considered positive if it caused a dose-related increase in the mean number of revertants per plate of at least one strain. This increase must be at least 2-fold in strains TA98 and TA100 and at least 3-fold in strains TA1535, TA1537, and TA1538.

C. COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality were provided.

[DICAMBA, DGA SALT] SALMONELLA/MAMMALIAN ACTIVATION; GENE MUTATION (84-2)

II. REPORTED RESULTS

A. Preliminary cytotoxicity assay: Ten doses of the test substance ranging from 6.7 to 5000 μg/plate were evaluated with and without S9 activation in single plate cultures using strain TA100. No compound precipitation or cytotoxicity was apparent at any of the nonactivated or S9-activated doses. Revertant colony counts were comparable to the vehicle controls.

B. Mutagenicity assay: Five doses of the test substance ranging from 100 to 5000 μg/plate were evaluated with and without S9 activation in triplicate plate cultures using strains TA98, TA100, TA1535, TA1537, or TA1538. The mutagenicity assays were performed in duplicate. Summary results from Tables 22 and 23 (study report pages 37-38) are appended to this DER. There were no significant differences in the number of revertant colonies in any tester strain at any dose level/condition in either the initial or repeat assays. Neither toxicity nor precipitate was observed in any tester strain with or without S9 activation in either assay. The positive control substances induced significant increases in revertant colonies in their respective strains. The vehicle controls responded in a similar manner to historical controls. Based on these results, the study authors concluded that the DGA salt of dicamba was not mutagenic in this microbial gene mutation assay.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. The reviewer agrees with the study authors' conclusions that the DGA salt of dicamba was assayed over an appropriate dose range and failed to induce a genotoxic response. Similarly, the sensitivity of the test system to detect mutagenesis was adequately demonstrated by the responses obtained with the nonactivated and S9-activated positive controls. This study is classified as acceptable.

B. Study deficiencies - None.

ATTACHMENTS

Salmonella Mutagenicity Assay
Summary of Results

Table 22

Test Article Id : DGA Salt of Dicamba
Study Number : TE237.501014 Experiment No : B1

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (μg)	TA98	TA100	TA1535	TA1537	TA1538
0.0	17 ± 4	151 ± 4	13 ± 3	6 ± 3	9 ± 1
100	15 ± 3	145 ± 16	12 ± 1	4 ± 2	10 ± 2
333	19 ± 3	142 ± 18	9 ± 4	7 ± 3	7 ± 1
1000	15 ± 6	151 ± 9	12 ± 4	6 ± 2	7 ± 3
3333	25 ± 6	159 ± 10	10 ± 2	5 ± 2	4 ± 2
5000	17 ± 4	157 ± 7	10 ± 4	6 ± 1	8 ± 2
Pos	276 ± 21	748 ± 59	542 ± 6	409 ± 73	470 ± 22

Liver Microsomes: Rat liver S9

Dose (μg)	TA98	TA100	TA1535	TA1537	TA1538
0.0	30 ± 1	159 ± 13	12 ± 1	6 ± 2	18 ± 1
100	27 ± 6	161 ± 25	12 ± 6	6 ± 1	15 ± 3
333	32 ± 4	166 ± 12	15 ± 6	7 ± 3	14 ± 4
1000	33 ± 9	153 ± 10	16 ± 6	8 ± 5	12 ± 4
3333	26 ± 4	166 ± 21	11 ± 6	7 ± 1	10 ± 4
5000	29 ± 3	128 ± 48	10 ± 3	6 ± 1	11 ± 3
Pos	822 ± 105	901 ± 58	117 ± 22	85 ± 4	822 ± 50

0.0 = Vehicle plating aliquot of 50 μl
Pos = Positive Control concentrations as specified in Materials and Methods section.

Salmonella Mutagenicity Assay
Summary of Results

Table 23

Test Article Id : DGA Salt of Dicamba
Study Number : TE237.501014 Experiment No : B2

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (µg)	TA98	TA100	TA1535	TA1537	TA1538
0.0	21 ± 9	162 ± 11	18 ± 2	9 ± 4	12 ± 3
100	21 ± 2	167 ± 12	19 ± 2	6 ± 1	10 ± 2
333	23 ± 7	153 ± 6	11 ± 1	8 ± 2	9 ± 4
1000	25 ± 3	162 ± 16	12 ± 5	5 ± 2	11 ± 3
3333	24 ± 2	188 ± 3	13 ± 7	8 ± 6	9 ± 1
5000	34 ± 2	177 ± 16	15 ± 5	7 ± 1	14 ± 6
Pos	270 ± 25	989 ± 38	783 ± 59	529 ± 151	481 ± 66

Liver Microsomes: Rat liver S9

Dose (µg)	TA98	TA100	TA1535	TA1537	TA1538
0.0	41 ± 2	174 ± 27	13 ± 5	5 ± 2	15 ± 1
100	33 ± 10	182 ± 19	17 ± 5	8 ± 1	12 ± 7
333	32 ± 2	181 ± 6	17 ± 3	9 ± 2	14 ± 3
1000	34 ± 2	177 ± 9	18 ± 5	8 ± 2	17 ± 3
3333	39 ± 3	175 ± 10	15 ± 4	9 ± 5	18 ± 3
5000	35 ± 5	173 ± 17	17 ± 6	9 ± 5	7 ± 3
Pos	1596 ± 246	1741 ± 169	171 ± 14	150 ± 26	1875 ± 17

0.0 = Vehicle plating aliquot of 50 µl
Pos = Positive Control concentrations as specified in Materials and Methods section.

DATA EVALUATION RECORD

AMINE SALTS OF DICAMBA-DGA (Diglycolamine Salt)

Study Type: 84-2; Mammalian Cells in Culture - Gene Mutation Assay in Mouse Lymphoma Cells

Work Assignment No. 1-15E (MRID 43310305)

Prepared for

Health Effects Division
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U.S. Environmental Protection Agency
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Prepared by

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Date: 4/10/96

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Reto Engler, Ph.D.

Signature: Reto Engler
Date: 4/10/96

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Jess Rowland, M.S Jess Rowland 7/2/96
Review Section II, Toxicology Branch II

EPA Secondary Reviewer: Yiannakis. Ioannou Ph.D 7/23/96
Review Section II, Toxicology Branch II

DATA EVALUATION RECORD

STUDY TYPE: Mammalian cells in culture gene mutation assay in mouse lymphoma cells

OPP Guideline Number: §84-2

DP BARCODE: D206005 SUBMISSION CODE: S470884

P.C. CODE: 128931 TOX. CHEM. NO.: **295F**

TEST MATERIAL (PURITY): Dicamba DGA salt (39.7% ai)

SYNONYMS: Diglycolamine salt of 3,6-dichloro-*o*-anisic acid

CITATION: San, R., and J. Clarke. (1994) L5178Y/TK$^{+/-}$ Mouse lymphoma mutagenesis assay with a confirmatory assay. Microbiological Associates, Inc., Rockville, MD. Laboratory Study Number TE237.701020. June 15, 1994. MRID 43310305. Unpublished.

SPONSOR: Sandoz Agro, Inc., Des Plaines, IL

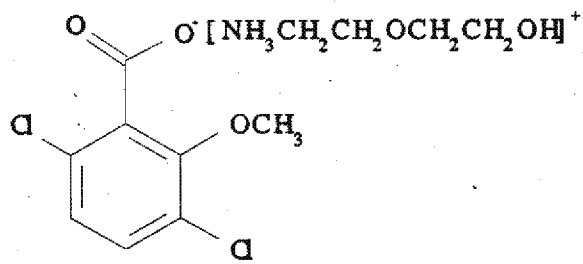
EXECUTIVE SUMMARY: In a mammalian cell gene mutation assay at the thymidine kinase locus (MRID 43310305), L5178Y mouse lymphoma cells cultured *in vitro* were exposed to dicamba DGA salt (39.7% ai) in distilled water at concentrations of 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, and 5000 μg/mL in the presence and absence of S9 mammalian metabolic activation. Dicamba DGA salt was tested up to the limit dose. Under nonactivation conditions, the % total growth values over the evaluated dose range were from 68-116% (initial assay) and 72-105% (confirmatory assay). The mutation frequencies (MFs) for all of the treated cultures were <2x the solvent controls. The S9-activation assay confirmed the findings of the nonactivation assay. The % total growth values were 43-102% (initial assay) and 46-99% (confirmatory assay). The MFs for all of the treated cultures were <2x the solvent controls with the exception of the 4500 μg/mL dose in the initial trial, which had a MF of approximately 2x background. However, this result was not reproducible. Therefore, it was determined that **dicamba DGA salt was not mutagenic under [redacted] nonactivation or S9-activation conditions.** In both the nonactivated and activated conditions, the positive controls induced the appropriate response.

This study is classified as **acceptable** and satisfies the guideline requirement for *in vitro* mutagenicity (mammalian forward gene mutation) data (§84-2).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Dicamba DGA salt
   Description: Caramel-colored viscous liquid
   Lot/Batch #: 5998-1
   Purity: 39.7% ai
   Stability of compound: Not provided
   CAS #: 104040-79-1
   Structure:



   Solvent used: Sterile distilled water
   Other comments: The test material was stored at room temperature, protected from light. Dosing solutions were prepared under amber lights and kept in darkness throughout the 4 hour exposure period. Actual concentrations of the low, mid, and high doses used in the initial and confirmatory mutagenicity assays were verified analytically by HPLC. The dosing solutions were from 92.4-101% of the nominal concentrations for the two replicate trials (study report page 26).

2. Control Materials
   Negative: The negative control was the test material solvent, water.
   Solvent/final concentration: Sterile, distilled, deionized water (1%, v/v)
   Positive:
   Nonactivation: Ethyl methanesulfonate was used at concentrations of 0.25 and 0.5 μL/mL with DMSO as the solvent.
   Activation: 7,12-Dimethylbenz(a)anthracene was used at concentrations of 2.5 and 5.0 μg/mL. The solvent was DMSO.

3. Activation

S-9 was derived from:

X	Aroclor 1254	X	Induced	X	Rat	X	Liver
	Phenobarbital		Non-induced		Mouse		Lung
	None				Hamster		Other
	Other				Other		

The S9 homogenate was prepared by the testing laboratory and contained DL-isocitric acid (11.25 mg), NADP (6.0 mg), Fischer's medium with 0.1% pluronics (0.75 mL), and S9 homogenate (0.25 mL); the pH was adjusted to 7.0 prior to the addition of the S9.

4. Test Cells

Mouse lymphoma L5178Y cells were used in the study.

Properly maintained? **Yes**
Periodically checked for mycoplasma contamination? **Not reported**
Periodically checked for karyotype stability? **Not reported**
Periodically "cleansed" against high spontaneous background? **Yes**

Media: Fischer's Medium for Leukemic Cells of Mice with 0.1% pluronic solution supplemented with heat-inactivated horse serum (10%, v:v) and 4mM L-glutamine

5. Locus Examined

Thymidine kinase (TK)
Selection agent: 3 μg/mL trifluorothymidine (TFT)

6. Test compound concentrations used

a. Preliminary Assays:

Nonactivated and activated conditions: Nine doses (0.5, 1.0, 5.0, 10, 50, 100, 500, 1000, and 5000 μg/mL) were tested with and without S9 activation. The suspension growth (Day 1 cell concentration/0.3×10^6 cells/mL) x (Day 2 cell concentration/Day 1 adjusted cell concentration) were determined as a measure of toxicity for all evaluated levels.

b. Mutation Assays:

Nonactivated and activated conditions: Ten doses (900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, and 5000 μg/mL) were tested with and without S9 activation. MFs were determined for all evaluated levels. Initial and confirmatory trials were assayed identically.

B. TEST PERFORMANCE

1. Cell treatment

a. Cells were exposed to the test compound, negative/solvent or positive controls for:
4 hours (nonactivated); 4 hours (activated)

b. After washing, cells were cultured for 2 days (expression period) before cell selection.

c. After expression, $1x10^6$ cells/dish (3 dishes/group) were cultured for 10-12 days in selection medium to determine numbers of mutants and 200 cells/dish (3 dishes/group) were cultured for 10-12 days without selective agent to determine cloning efficiency.

2. Statistical Methods
The data were not evaluated for statistical significance.

3. Evaluation Criteria

a. Assay validity: The assay was considered valid if (i) the mutant frequencies (MFs) for the positive controls is ≥2x the solvent controls, (ii) the spontaneous MFs (for the solvent controls) must be from 20 to 100 per 10^6 cells, (iii) the cloning efficiency of the solvent controls must be >50%.

b. Positive result: The test material was considered mutagenic if it caused a dose-related increase in the MFs with at least two dose levels in the ≥10% total growth range demonstrating MFs that are 2-fold greater than background. Alternatively, the test material was considered mutagenic if it caused a reproducible 2-fold increase for at least one dose level.

C. COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

II. REPORTED RESULTS

A. Preliminary cytotoxicity assay

Nine preliminary doses of the dicamba DGA salt (0.5, 1.0, 5.0, 10, 50, 100, 500, 1000, and 5000 μg/mL) were evaluated with and without S9 activation. Suspension growths were determined as a measure of toxicity for all evaluated levels. The test material was soluble at all dose levels. No toxicity was observed in any of the treated cultures assayed without S9 activation. For the activation conditions, the suspension growth was depressed by 36% relative to the solvent controls at 5000 μg/mL. Based on these results, the mutation assays were conducted with ten doses (900-5000 μg/mL) with and without S9 activation.

B. Mutagenicity assay

The test material was soluble at all dose levels evaluated with and without S9 activation (900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, and 5000 μg/mL). Initial and confirmatory trials were assayed at these dose levels. Under both the activation and nonactivation conditions for the initial and confirmatory trials, all of the following criteria for assay validity were met: (i) the mutant frequencies (MFs) for the positive controls were $\geq$2x the solvent controls, (ii) the spontaneous MFs (for the solvent controls) were from 20 to 100 per 10^6 cells, and (iii) the cloning efficiency of the solvent controls were >50%.

Each trial included three cultures per dose level, four solvent controls (water) for the test article, two solvent controls (DMSO) for the positive controls, and two concentrations of the positive control. Results from the initial and confirmatory trials of the mutation assay are presented in Appendices 1-4 (Tables 2, 4, 6, and 8; study report pages 15, 17, 19, and 21, respectively) included in this DER and summarized as follows:

Nonactivation conditions: The % total growth values [(% suspension growth x % cloning growth)/100] over the evaluated dose range were from 68-116% for the initial assay and 72-105% for the confirmatory assay. The MFs for the treated cultures were 25-60 per 10^6 cells for the initial assay (Table 2, study report page 15) and 26-45 per 10^6 cells for the confirmatory assay (Table 6, study report page 19). The average MFs for the solvent controls were 35 and 37 per 10^6 cells for the initial and confirmatory assays, respectively. All MFs for the cultures treated with dicamba DGA salt were <2x the background. Therefore, it was determined that dicamba DGA salt was not mutagenic under the nonactivation conditions.

S9-activation conditions: The % total growth values over the evaluated dose range were from 43-102% for the initial assay and 46-99% for the confirmatory assay. The MFs for the treated cultures were 28-64 per 10^6 cells for the initial assay (Table 4, study report page 17) and 70-109 per 10^6 cells for the confirmatory assay (Table 8, study report page 21). The average MFs for the solvent controls were 32 and 63 per 10^6 cells for the initial and confirmatory assays, respectively. A 2-fold increase in the MFs was reported for the 4500 μg/mL dose (64 vs. 32 per 10^6 cells for the background, initial trial); however, it was not reproducible. All other MFs for the treated cultures were <2x the background. Therefore, it was determined that dicamba DGA salt was not a mutagen under the S9-activation conditions.

III. DISCUSSION/CONCLUSIONS

A. Investigator's Conclusions

The study authors concluded that, under the conditions of this study, dicamba DGA salt was not mutagenic in the presence or absence of metabolic activation.

B. Reviewer's Discussion

We agree with the study authors' conclusion that dicamba DGA salt was not mutagenic under the conditions of this study. The dose levels tested were adequate for both the nonactivation and activation conditions as it was tested to the limit concentration. The positive controls induced MFs that were >2x the background. Under the S9 activated condition, one dose level in the initial trial induced MFs approximately 2x the solvent controls. However, because it was not reproducible and all other MFs for the treated cultures were <2x the background, dicamba DGA salt was not considered mutagenic in this assay.

IV. STUDY DEFICIENCIES

The percent of S9 homogenate in the S9 cofactor mix was 25%. This exceeds the EPA recommended level of 4-10%. However, as excessive toxicity was not observed, it does not alter the conclusions of the study.

APPENDIX 1

TABLE 2

CLONING DATA FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS
TREATED WITH DGA Salt of Dicamba
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION
INITIAL ASSAY

Test Article Concentration (µg/ml)	Ave #/ TFT Plate[a]	TFT Stand Dev	Ave #/ V.C. Plate[a]	V.C. Stand Dev	Mutant Frequency[b]	Induced Mutant Frequency[c]	% Total Growth[d]
5000	32/3	5	106/3	0	60	25	68
4500	19/3	3	100/3	0	38	3	71
4000	18/3	1	124/3	14	29	-6	92
3500	22/3	3	130/3	4	34	-1	96
3000	23/3	3	118/3	9	39	4	109
2500	20/3	2	130/3	13	31	-4	97
2000	21/3	3	112/3	8	38	3	83
1500	23/3	4	125/3	9	37	2	96
1000	26/3	2	146/3	7	36	1	116
900	18/3	1	144/3	11	25	-10	114
Solvent 1	19/3	1	128/3	7	30		
Solvent 2	19/3	5	129/3	5	29		
Solvent 3	31/3	4	130/3	7	48		
Solvent 4	21/3	3	131/3	7	32		
Mean Solvent Mutant Frequency= 35							
Positive Control - Ethyl Methanesulfonate (µl/ml)							
0.50	334/3	1	61/3	1	1095	1057	22
0.25	224/3	14	103/3	2	435	397	53
Solvent 1	26/3	1	148/3	6	35		
Solvent 2	24/3	2	121/3	15	40		
Mean Solvent Mutant Frequency= 38							

[a] - Average # of colonies per plate and # of plates scored

[b] - Mutant frequency (per 10^6 surviving cells) = (Average # TFT colonies / average # VC colonies) x 200

[c] - Induced mutant frequency (per 10^6 surviving cells) = mutant frequency - average mutant frequency of solvent controls

[d] - % total growth = (% suspension growth x % cloning growth) / 100

APPENDIX 2

TABLE 4

CLONING DATA FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS
TREATED WITH DGA Salt of Dicamba
IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION
INITIAL ASSAY

Test Article Concentration (µg/ml)	Ave #/ TFT Plate[a]	TFT Stand Dev	Ave #/ V.C. Plate[a]	V.C. Stand Dev	Mutant Frequency[b]	Induced Mutant Frequency[c]	% Total Growth[d]
5000	36/2	2	137/3	1	53	21	43
4500	34/3	7	106/3	5	64	32	53
4000	38/3	6	120/3	13	63	31	61
3500	34/3	1	112/3	6	61	29	68
3000	30/3	6	132/3	9	45	13	89
2500	23/3	3	121/3	13	38	6	79
2000	24/3	3	125/3	3	38	6	89
1500	24/3	3	144/3	9	33	1	102
1000	20/3	2	141/3	5	28	-4	100
900	24/3	5	131/3	12	37	5	94
Solvent 1	24/3	5	129/3	12	37		
Solvent 2	21/3	4	146/3	8	29		
Solvent 3	20/3	3	137/3	9	29		
Solvent 4	20/3	3	122/3	7	33		
Mean Solvent Mutant Frequency= 32							
Positive Control - 7,12 Dimethylbenz(a)anthracene (µg/ml)							
5.0	88/3	9	24/3	5	733	690	4
2.5	127/3	17	85/3	15	299	256	50
Solvent 1	25/3	2	115/3	7	43		
Solvent 2	23/3	4	108/3	6	43		
Mean Solvent Mutant Frequency= 43							

[a] - Average # of colonies per plate and # of plates scored

[b] - Mutant frequency (per 10^6 surviving cells) = (Average # TFT colonies / average # VC colonies) x 200

[c] - Induced mutant frequency (per 10^6 surviving cells) = mutant frequency - average mutant frequency of solvent controls

[d] - % total growth = (% suspension growth x % cloning growth) / 100

APPENDIX 3

TABLE 6

CLONING DATA FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS
TREATED WITH DGA Salt of Dicamba
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION
CONFIRMATORY ASSAY

Test Article Concentration (µg/ml)	Ave #/ TFT Plate[a]	TFT Stand Dev	Ave #/ V.C. Plate[a]	V.C. Stand Dev	Mutant Frequency[b]	Induced Mutant Frequency[c]	% Total Growth[d]
5000	18/3	2	111/3	2	32	-5	81
4500	19/3	6	88/3	5	43	6	72
4000	14/3	1	106/3	12	26	-11	93
3500	16/3	5	101/3	1	32	-5	90
3000	24/3	3	107/3	5	45	8	96
2500	19/3	5	102/3	2	37	0	95
2000	17/3	2	121/3	12	28	-9	105
1500	15/3	3	88/3	6	34	-3	85
1000	16/3	3	112/2	2	29	-8	99
900	15/3	3	102/3	1	29	-8	96
Solvent 1	19/3	1	108/3	3	35		
Solvent 2	21/3	5	109/3	11	39		
Solvent 3	23/3	3	114/3	12	40		
Solvent 4	15/3	1	93/3	7	32		
Mean Solvent Mutant Frequency= 37							
Positive Control - Ethyl Methanesulfonate (µl/ml)							
0.50	241/3	10	40/3	10	1205	1173	20
0.25	217/3	4	83/3	5	523	491	58
Solvent 1	19/3	3	109/3	8	35		
Solvent 2	14/3	2	101/3	13	28		
Mean Solvent Mutant Frequency= 32							

[a] - Average # of colonies per plate and # of plates scored

[b] - Mutant frequency (per 10^6 surviving cells) = (Average # TFT colonies / average # VC colonies) x 200

[c] - Induced mutant frequency (per 10^6 surviving cells) = mutant frequency - average mutant frequency of solvent controls

[d] - % total growth = (% suspension growth x % cloning growth) / 100

APPENDIX 4

TABLE 8

CLONING DATA FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS
TREATED WITH DGA Salt of Dicamba
IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION
CONFIRMATORY ASSAY

Test Article Concentration (µg/ml)	Ave #/ TFT Plate[a]	TFT Stand Dev	Ave #/ V.C. Plate[a]	V.C. Stand Dev	Mutant Frequency[b]	Induced Mutant Frequency[c]	% Total Growth[d]
5000	54/3	10	117/3	17	92	29	48
4500	54/3	0	110/3	6	98	35	46
4000	58/3	11	132/3	14	88	25	65
3500	58/3	3	121/3	16	96	33	66
3000	71/3	2	130/3	6	109	46	75
2500	58/3	4	124/3	5	94	31	71
2000	57/3	3	108/3	3	106	43	68
1500	52/3	4	148/3	11	70	7	99
1000	49/3	6	131/3	6	75	12	85
900	54/3	3	143/3	5	76	13	90
Solvent 1	44/3	4	148/3	8	59		
Solvent 2	51/3	5	145/3	4	70		
Solvent 3	39/3	3	133/3	6	59		
Solvent 4	39/3	2	122/3	7	64		
Mean Solvent Mutant Frequency= 63							
Positive Control - 7,12 Dimethylbenz(a)anthracene (µg/ml)							
5.0	88/3	10	67/3	10	263	197	28
2.5	88/3	10	87/3	5	202	136	51
Solvent 1	42/3	6	127/3	3	66		
Solvent 2	46/3	2	139/3	3	66		
Mean Solvent Mutant Frequency= 66							

[a] - Average # of colonies per plate and # of plates scored

[b] - Mutant frequency (per 10^6 surviving cells) = (Average # TFT colonies / average # VC colonies) x 200

[c] - Induced mutant frequency (per 10^6 surviving cells) = mutant frequency - average mutant frequency of solvent controls

[d] - % total growth = (% suspension growth x % cloning growth) / 100

DATA EVALUATION REPORT

ISOPROPYLAMINE SALT OF DICAMBA

Study Type: 84-2; *Salmonella typhimurium*/Mammalian Activation Gene Mutation Assay

Dynamac Study No. 115C (MRID 43310303)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticides Health Effects Group
Sciences Division
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Date: 1/19/96

Quality Assurance:
Reto Engler, Ph.D.

Signature: Reto Engler by WJS
Date: 1/19/96

Disclaimer

This Data Evaluation Report may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

[DICAMBA, IPA SALT] SALMONELLA/MAMMALIAN ACTIVATION; GENE MUTATION (84-2)

EPA Reviewer: Jess Rowland, M.S. Jess Rowland 7/2/96
Review Section II, Toxicology Branch II (7509C)

EPA Secondary Reviewer: Yiannakis Ioannou, Ph.D JMI 7/23/96
Review Section II, Toxicology Branch II (7509C)

DATA EVALUATION RECORD

STUDY TYPE: *Salmonella*/mammalian activation gene mutation assay

OPP Guideline Number: §84-2

DP BARCODE: D206005 SUBMISSION CODE: S470884

P.C. CODE: 128944 TOX. CHEM. NO.: **295G**

TEST MATERIAL (PURITY): Isopropylamine salt of dicamba (32.3% active ingredient)

SYNONYMS: IPA salt of dicamba

CITATION: San, R., and D. Pugh (1994) *Salmonella* Plate Incorporation Mutagenicity Assay (Ames Test) with Confirmatory Assay. Microbiological Associates, Inc., Rockville, MD. Study #. TE238.501014. June 29, 1994. **MRID No. 43310303.** Unpublished.

SPONSOR: Sandoz Agro, Inc., Des Plaines, IL

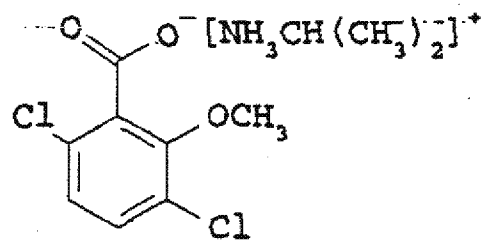
EXECUTIVE SUMMARY: In a microbial mutagenicity assay (MRID 43310303), *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, or TA1538 were exposed to the IPA salt of dicamba (32.3% a.i.) in deionized distilled water at concentrations of 100, 333, 1000, 3333, or 5000 μg/plate in the presence and absence of mammalian metabolic activation. Preparations for metabolic activation were made from induced rat livers.

The IPA salt of dicamba was tested up to the limit concentration of 5000 μg/plate and no cytotoxicity was observed. The positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background (reversion to prototrophy).**

This study is classified as **acceptable**, and satisfies the requirements for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

A. MATERIALS

1. Test Material: IPA salt of dicamba
   Description: Caramel color viscous liquid
   Lot/Batch #: 5998-3
   Purity: 32.3% a.i.
   Stability of compound: Not reported
   CAS No. 55871-02-8



Solvent used: Deionized distilled water (DDW)
Other comments: The test material was stored at room temperature and protected from light. Dosing solutions were prepared on the days of testing and aliquots of the low, mid, and high dose were analyzed by HPLC to confirm the nominal concentrations. The dosing solutions were 82-103% of the nominal concentrations.

2. Control Materials:
   Negative: DDW
   Solvent/final concentration: DDW/50 µL per plate
   Positive: Nonactivation:
   2-Nitrofluorene 1.0 µg/plate TA98, TA1538
   9-Aminoacridine 75.0 µg/plate TA1537
   Sodium azide 1.0 µg/plate TA100, TA 1535

   Activation:
   2-Aminoanthracene (2-anthramine) 1.0 µg/plate all strains

3. Activation: S9 derived from

X Aroclor 1254	X induced	X rat	X liver
__ phenobarbital	__ non-induced	__ mouse	__ lung
__ none		__ hamster	__ other
__ other			__ other

S9 mix composition: The S9 was prepared and stored frozen (≤-70 C) until use. The S9 mix was prepared immediately prior to use and contained: S9 fraction (10% v/v), $MgCl_2$ (8 mM), KCl (33 mM), NADP (4 mM), glucose-6-phosphate (5 mM), and phosphate buffer (100 mM); 0.5 mL of S9 was mix used per culture flask.

4. Test organisms: S. typhimurium strains

__ TA97	X TA98	X TA100	__ TA102	__ TA104
X TA1535	X TA1537	X TA1538		

Properly maintained? Yes
Checked for appropriate genetic markers (rfa mutation, R factor)? Yes

5. Test compound concentrations used:

Preliminary cytotoxicity test: Ten dose levels (6.7, 10, 33, 67, 100, 333, 667, 1000, 3333, or 5000 μg/plate) were evaluated with strain TA100 in the presence and absence of S9 activation; single plates were used per dose, per condition; vehicle controls were included.

Mutagenicity assay: Five dose levels (100, 333, 1000, 3333, or 5000 μg/plate) were evaluated with strains TA98, TA100, TA1535, TA1537, or TA1538 in the presence and absence of S9 activation; triplicate plates were used for each dose, strain, and condition; vehicle and positive control groups were included. A confirmatory assay was also performed.

B. TEST PERFORMANCE

1. Type of Salmonella assay:
   - X standard plate test
   - __ pre-incubation (__ minutes)
   - __ "Prival" modification (i.e. azo-reduction method)
   - __ spot test
   - __ other

2. Protocol: Tester strains were inoculated into nutrient broth culture approximately 12 hours prior to dosing and incubated at 37 ± 2 C. Test substance and positive control substances were diluted in DDW to specified concentrations. Bacteria (100 μL), 50 μL of DDW, test substance, or positive control, and 0.5 mL of S9 mix were added to glass tubes containing 2 mL of melted top agar. The mixture was vortexed and poured on plates containing a layer of minimal agar medium. After the top agar solidified, the plates were inverted and incubated at 37 ± 2 C for approximately 48-

72 hours. The plates were evaluated for gross toxic effects and total revertant colony numbers. Revertant colonies were counted either entirely by hand or by an automatic colony counter. The means and standard deviations for the mutation tests were determined from the counts of triplicate plates per strain, per dose, per condition.

3. Evaluation Criteria

(a) Assay validity: The assay was considered acceptable if (1) the appropriate genetic markers were verified for each tester strain, (2) the number of spontaneous revertants for each tester strain was within specified limits, (3) the density of the tester strain cultures was $\geq 3x10^8$ cells/mL, and (4) the nonactivated and S9-activated positive controls induced at least a tripling of the number of revertants compared with the solvent controls.

(b) Positive response: The test material was considered positive if it caused a dose-related increase in the mean number of revertants per plate of at least one strain. This increase must be at least 2-fold in strains TA98 and TA100 and at least 3-fold in strains TA1535, TA1537, and TA1538.

d. COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

II. REPORTED RESULTS

A. Preliminary cytotoxicity assay: Ten doses of the test substance ranging from 6.7 to 5000 μg/plate were evaluated with and without S9 activation in single plate cultures using strain TA100. No compound precipitation or cytotoxicity was apparent at any of the nonactivated or S9-activated doses. Revertant colony counts were comparable to the vehicle controls.

B. Mutagenicity assay: Five doses of the test substance ranging from 100 to 5000 μg/plate were evaluated with and without S9 activation in triplicate plate cultures using strains TA98, TA100, TA1535, TA1537, or TA1538. The mutagenicity assays were performed in duplicate. Summary results from Tables 22 and 23 (study report pages 37-38) are appended to this DER. There were no significant differences in the number of revertant colonies in any tester strain at any dose level/condition in either the initial or repeat assays. Neither toxicity nor precipitate was observed in any tester strain with or without S9 activation in either assay. The positive control substances induced significant increases in revertant colonies in their respective strains. The vehicle controls responded in a similar manner to historical controls. Based on these results, the study authors concluded that the IPA salt of dicamba was not mutagenic in this microbial gene mutation assay.

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. The reviewer agrees with the study authors' conclusions that the IPA salt of dicamba was assayed over an appropriate dose range and failed to induce a genotoxic response. Similarly, the sensitivity of the test system to detect mutagenesis was adequately demonstrated by the responses obtained with the nonactivated and S9-activated positive controls. This study is classified as acceptable.

B. Study deficiencies - None.

ATTACHMENTS

Salmonella Mutagenicity Assay
Summary of Results

Table 22

Test Article Id : IPA Salt of Dicamba
Study Number : TE238.501014 Experiment No : B1

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (μg)	TA98	TA100	TA1535	TA1537	TA1538
0.0	18 ± 3	164 ± 14	14 ± 5	7 ± 5	11 ± 5
100	21 ± 3	157 ± 11	14 ± 2	6 ± 2	6 ± 5
333	21 ± 8	154 ± 5	13 ± 4	4 ± 2	8 ± 1
1000	21 ± 5	163 ± 7	14 ± 2	7 ± 4	6 ± 4
3333	19 ± 3	174 ± 5	13 ± 4	7 ± 1	9 ± 3
5000	16 ± 4	167 ± 2	12 ± 3	5 ± 3	9 ± 5
Pos	307 ± 11	1007 ± 16	692 ± 26	532 ± 181	581 ± 65

Liver Microsomes: Rat liver S9

Dose (μg)	TA98	TA100	TA1535	TA1537	TA1538
0.0	28 ± 4	186 ± 13	15 ± 1	9 ± 3	14 ± 3
100	27 ± 3	194 ± 8	16 ± 1	10 ± 1	15 ± 2
333	30 ± 6	180 ± 14	17 ± 3	8 ± 6	14 ± 2
1000	31 ± 4	166 ± 11	12 ± 4	9 ± 5	12 ± 4
3333	28 ± 5	190 ± 11	14 ± 4	7 ± 2	20 ± 7
5000	27 ± 1	167 ± 27	16 ± 3	7 ± 4	14 ± 1
Pos	1733 ± 170	1491 ± 172	169 ± 9	105 ± 32	1402 ± 58

0.0 = Vehicle plating aliquot of 50 μl
Pos = Positive Control concentrations as specified in Materials and Methods section.

Salmonella Mutagenicity Assay
Summary of Results

Table 23

Test Article Id : IPA Salt of Dicamba
Study Number : TE238.501014 Experiment No : B2

Average Revertants Per Plate ± Standard Deviation

Liver Microsomes: None

Dose (μg)	TA98	TA100	TA1535	TA1537	TA1538
0.0	16 ± 2	145 ± 10	6 ± 3	8 ± 3	6 ± 3
100	20 ± 4	156 ± 10	10 ± 2	7 ± 3	5 ± 1
333	17 ± 2	163 ± 9	12 ± 1	5 ± 3	8 ± 3
1000	18 ± 3	138 ± 6	7 ± 7	5 ± 4	6 ± 1
3333	15 ± 3	149 ± 16	9 ± 5	6 ± 2	7 ± 5
5000	20 ± 3	145 ± 9	13 ± 1	8 ± 3	5 ± 5
Pos	228 ± 23	759 ± 181	441 ± 40	553 ± 180	432 ± 22

Liver Microsomes: Rat liver S9

Dose (μg)	TA98	TA100	TA1535	TA1537	TA1538
0.0	27 ± 1	166 ± 12	18 ± 2	7 ± 2	12 ± 0
100	24 ± 2	177 ± 14	17 ± 3	9 ± 2	15 ± 1
333	29 ± 4	170 ± 22	10 ± 1	6 ± 2	10 ± 3
1000	25 ± 4	163 ± 12	19 ± 2	6 ± 2	11 ± 4
3333	29 ± 2	162 ± 8	15 ± 2	6 ± 3	11 ± 4
5000	31 ± 6	167 ± 6	12 ± 1	7 ± 2	13 ± 2
Pos	1444 ± 21	1979 ± 851	169 ± 39	123 ± 39	1420 ± 281

0.0 = Vehicle plating aliquot of 50 μl
Pos = Positive Control concentrations as specified in Materials and Methods section.

012000

DATA EVALUATION RECORD

AMINE SALTS OF DICAMBA-IPA (Isopropylamine Salt)

Study Type: 84-2; Mammalian Cells in Culture - Gene Mutation Assay in Mouse Lymphoma Cells

Work Assignment No. 1-15F (MRID 43310306)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

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Signature: Reto Engler
Date: 4/10/96

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Jess Rowland, M.S Jess Rowland 7/2/96
Review Section II, Toxicology Branch II

EPA Secondary Reviewer: Yiannakis Ioannou, Ph. J.M.I 7/23/96
Review Section II, Toxicology Branch (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Mammalian cells in culture gene mutation assay in mouse lymphoma cells

OPP Guideline Number: §84-2

DP BARCODE: D206005 SUBMISSION CODE: S470884

P.C. CODE: 128944 TOX. CHEM. NO.: **295G**

TEST MATERIAL (PURITY): Dicamba IPA salt (32.3% ai)

SYNONYMS: Isopropyl amine salt of 3,6-dichloro-*o*-anisic acid

CITATION: San, R., and J. Clarke. (1994) L5178Y/TK$^{+/-}$ Mouse lymphoma mutagenesis assay with a confirmatory assay. Microbiological Associates, Inc., Rockville, MD. Laboratory Study # TE238.701020. June 16, 1994. **MRID No. 43310306.** Unpublished.

SPONSOR: Sandoz Agro, Inc., Des Plaines, IL

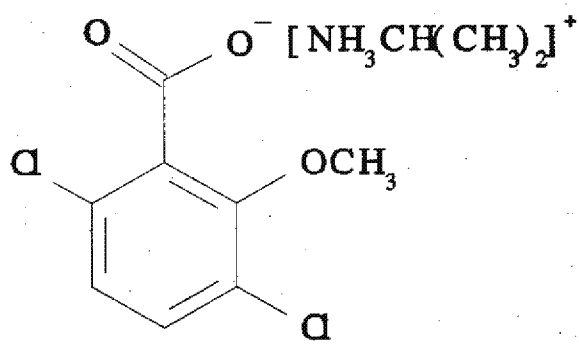
EXECUTIVE SUMMARY: In a mammalian cell gene mutation assay at the thymidine kinase locus (MRID 43310306), L5178Y mouse lymphoma cells cultured *in vitro* were exposed to dicamba IPA salt (32.3% ai) in distilled water at concentrations of 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, and 5000 μg/mL in the presence and absence of S9 mammalian metabolic activation. Dicamba IPA salt was tested up to the limit dose. Under nonactivation conditions, the % total growth values over the evaluated dose range were from 92-101% (initial assay) and 51-107% (confirmatory assay). The mutation frequencies (MFs) for all of the treated cultures were <2x the solvent controls. The S9-activation assay confirmed the findings of the nonactivation assay. The % total growth values were 75-126% (initial assay) and 49-114% (confirmatory assay). The MFs for all of the treated cultures were <2x the solvent controls. Therefore, it was determined that **dicamba IPA salt was not mutagenic under either nonactivation or S9-activation conditions.** In both the nonactivated and activated conditions, the positive controls induced the appropriate response.

This study is classified as **acceptable** and satisfies the guideline requirement for **in vitro** mutagenicity (mammalian forward gene mutation) data (§84-2).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Dicamba IPA salt
Description: Caramel color viscous liquid
Lot/Batch #: 5998-3
Purity: 32.3% ai
Stability of compound: Not provided
CAS #: 55871-02-8
Structure:



Solvent used: Sterile distilled water
Other comments: The test material was stored at room temperature, protected from light. Dosing solutions were prepared under amber lights and kept in darkness throughout the 4 hour exposure period. Actual concentrations of the low, mid, and high doses used in the initial and confirmatory mutagenicity assays were verified analytically by HPLC. The dosing solutions were from 95.9-101% of the nominal concentrations for the two replicate trials (study report page 26), with the exception of the mid dose used in the confirmatory trial which was 77.1% of the nominal concentration. The study authors did not consider this single aberration to adversely affect the outcome of the study as all other doses were within ±5% of theoretical.

2. Control Materials
Negative: The negative control was the test material solvent, water.
Solvent/final concentration: Sterile, distilled, deionized water (1%, v/v)
Positive:
Nonactivation: Ethyl methanesulfonate was used at concentrations of 0.25 and 0.5 μL/mL with DMSO as the solvent.
Activation: 7,12-Dimethylbenz(a)anthracene was used at concentrations of 2.5 and 5.0 μg/mL. The solvent was DMSO.

3. Activation

S-9 was derived from:

X	Aroclor 1254	X	Induced	X	Rat	X	Liver
	Phenobarbital		Non-induced		Mouse		Lung
	None				Hamster		Other
	Other				Other		

The S9 homogenate was prepared by the testing laboratory and contained DL-isocitric acid (11.25 mg), NADP (6.0 mg), Fischer's medium with 0.1% pluronics (0.75 mL), and S9 homogenate (0.25 mL); the pH was adjusted to 7.0 prior to the addition of the S9.

4. Test Cells

Mouse lymphoma L5178Y cells were used in the study.

Properly maintained? **Yes**
Periodically checked for mycoplasma contamination? **Not reported**
Periodically checked for karyotype stability? **Not reported**
Periodically "cleansed" against high spontaneous background? **Yes**

Media: Fischer's Medium for Leukemic Cells of Mice with 0.1% pluronic solution supplemented with heat-inactivated horse serum (10%, v/v) and 4mM L-glutamine

5. Locus Examined

Thymidine kinase (TK)
Selection agent: 3 μg/mL trifluorothymidine (TFT)

6. Test compound concentrations used

a. Preliminary Assays:

Nonactivated and activated conditions: Nine doses (0.5, 1.0, 5.0, 10, 50, 100, 500, 1000, and 5000 μg/mL) were tested with and without S9 activation. The suspension growth (Day 1 cell concentration/0.3×10^6 cells/mL) x (Day 2 cell concentration/Day 1 adjusted cell concentration) were determined as a measure of toxicity for all evaluated levels.

b. Mutation Assays:

Nonactivated and activated conditions: Ten doses (900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, and 5000 μg/mL) were tested with and without S9 activation. MFs were determined for all evaluated levels. Initial and confirmatory trials were assayed identically.

B. TEST PERFORMANCE

1. Cell treatment

a. Cells exposed to test compound, negative/solvent or positive controls for: 4 hours (nonactivated); 4 hours (activated)

b. After washing, cells were cultured for 2 days (expression period) before cell selection.

c. After expression, $1x10^6$ cells/dish (3 dishes/group) were cultured for 10-12 days in selection medium to determine numbers of mutants, and 200 cells/dish (3 dishes/group) were cultured for 10-12 days without selective agent to determine cloning efficiency.

2. Statistical Methods

The data were not evaluated for statistical significance.

3. Evaluation Criteria

a. Assay validity: The assay was considered valid if (i) the mutant frequencies (MFs) for the positive controls is $\geq$2x the solvent controls, (ii) the spontaneous MFs (for the solvent controls) must be from 20 to 100 per 10^6 cells, and (iii) the cloning efficiency of the solvent controls must be $>50\%$.

b. Positive result: The test material was considered mutagenic if it caused a dose-related increase in the MFs with at least two dose levels in the $\geq 10\%$ total growth range demonstrating MFs that are 2-fold greater than background. Alternatively, the test material was considered mutagenic if it caused a reproducible 2-fold increase for at least one dose level.

C. COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

II. REPORTED RESULTS

A. Preliminary cytotoxicity assay

Nine preliminary doses of the dicamba IPA salt (0.5, 1.0, 5.0, 10, 50, 100, 500, 1000, and 5000 μg/mL) were evaluated with and without S9 activation. Suspension growths were determined as a measure of toxicity for all evaluated levels. The test material was soluble at all dose levels. Suspension growths were depressed by 5% (-S9) and by 16% (+S9) relative to the solvent controls at the 5000 μg/mL. Based on these results, the mutation assays were conducted with ten doses (900-5000 μg/mL) with and without S9 activation.

B. Mutagenicity assay

The test material was soluble at all dose levels evaluated with and without S9 activation (900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, and 5000 μg/mL). Initial and confirmatory trials were assayed at these dose levels. Under both the activation and nonactivation conditions for the initial and confirmatory trials, all of the following criteria for assay validity were met: (i) the mutant frequencies (MFs) for the positive controls were ≥2x the solvent controls, (ii) the spontaneous MFs (for the solvent controls) were from 20 to 100 per 10^6 cells, and (iii) the cloning efficiency of the solvent controls were >50%.

Each trial included three cultures per dose level, four solvent controls (water) for the test article, two solvent controls (DMSO) for the positive controls, and two concentrations of the positive control. Results from the initial and confirmatory trials of the mutation assay are presented in Appendices 1-4 (Tables 2, 4, 6, and 8; study report pages 15, 17, 19, and 21, respectively) included in this DER and summarized as follows:

Nonactivation conditions: The % total growth values [(% suspension growth x % cloning growth)/100] over the evaluated dose range were from 92-101% for the initial assay and 51-107% for the confirmatory assay. The MFs for the treated cultures were 21-31 per 10^6 cells for the initial assay (Table 2, study report page 15) and 23-50 per 10^6 cells for the confirmatory assay (Table 6, study report page 19). The average MFs for the solvent controls were 27 and 41 per 10^6 cells for the initial and confirmatory assays, respectively. All MFs for the cultures treated with dicamba IPA salt were <2x the background. Therefore, it was determined that dicamba IPA salt was not mutagenic under the nonactivation conditions.

S9-activation conditions: The % total growth values over the evaluated dose range were from 75-126% for the initial assay and 49-114% for the confirmatory assay. The MFs for the treated cultures were 26-58 per 10^6 cells for the initial assay (Table 4, study report page 17) and 50-85 per 10^6 cells for the confirmatory assay (Table 8, study report page 21). The average MFs for the solvent controls were 38 and 54 per 10^6 cells for the initial and confirmatory assays, respectively. The MFs for the all treated cultures were $<2x$ the background. Therefore, it was determined that dicamba IPA salt was not a mutagen under the S9-activation conditions.

III. DISCUSSION/CONCLUSIONS

A. Investigator's Conclusions

The study authors concluded that, under the conditions of this study, dicamba IPA salt was not mutagenic in the presence or absence of metabolic activation.

B. Reviewer's Discussion

We agree with the author's conclusion that dicamba IPA salt was not mutagenic under the conditions of this study. The dose levels tested were adequate for both the nonactivation and activation conditions as it was tested to the limit dose (5000 μg/mL). The positive controls induced MFs that were $>2x$ the background. The MFs for the all treated cultures were $<2x$ the background under both the nonactivation and activation conditions.

IV. STUDY DEFICIENCIES

One minor deficiency was that the mid dose level of the confirmatory mutagenicity assay was determined to be 77.1% of the nominal concentration. However, as all of the other assayed dosing solutions were from 95.9-101% of the nominal concentrations for the two replicate trials, this single aberration does not affect the acceptability of the study.

APPENDIX 1

TABLE 2

CLONING DATA FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS
TREATED WITH IPA Salt of Dicamba
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION
INITIAL ASSAY

Test Article Concentration (µg/ml)	Ave #/ TFT Plate[a]	TFT Stand Dev	Ave #/ V.C. Plate[a]	V.C. Stand Dev	Mutant Frequency[b]	Induced Mutant Frequency[c]	% Total Growth[d]
5000	15/3	3	134/3	9	22	-5	92
4500	23/3	3	148/3	14	31	4	101
4000	20/3	3	147/3	11	27	0	98
3500	22/3	4	142/3	1	31	4	93
3000	14/3	1	135/3	3	21	-6	92
2500	19/3	3	143/3	1	27	0	97
2000	18/3	5	133/3	7	27	0	99
1500	21/3	4	136/3	1	31	4	101
1000	19/3	3	127/3	6	30	3	97
900	18/3	2	134/3	6	27	0	98
Solvent 1	16/3	3	133/3	2	24		
Solvent 2	16/3	2	151/3	1	21		
Solvent 3	20/3	2	126/3	10	32		
Solvent 4	19/2	5	123/3	6	31		
Mean Solvent Mutant Frequency= 27							
Positive Control - Ethyl Methanesulfonate (µl/ml)							
0.50	389/3	7	82/3	9	949	917	35
0.25	263/3	23	106/3	5	496	464	57
Solvent 1	21/3	1	142/3	9	30		
Solvent 2	22/3	2	135/3	3	33		
Mean Solvent Mutant Frequency= 32							

[a] - Average # of colonies per plate and # of plates scored

[b] - Mutant frequency (per 10^6 surviving cells) = (Average # TFT colonies / average # VC colonies) x 200

[c] - Induced mutant frequency (per 10^6 surviving cells) = mutant frequency - average mutant frequency of solvent controls

[d] - % total growth = (% suspension growth x % cloning growth) / 100

APPENDIX 2

TABLE 4

CLONING DATA FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS TREATED WITH IPA Salt of Dicamba IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION INITIAL ASSAY

Test Article Concentration (µg/ml)	Ave #/ TFT Plate[a]	TFT Stand Dev	Ave #/ V.C. Plate[a]	V.C. Stand Dev	Mutant Frequency[b]	Induced Mutant Frequency[c]	% Total Growth[d]
5000	28/3	3	122/3	11	46	8	75
4500	37/3	1	128/3	8	58	20	83
4000	31/3	2	134/3	4	46	8	85
3500	28/3	2	129/3	4	43	5	88
3000	34/3	8	128/3	7	53	15	84
2500	26/3	4	129/3	5	40	2	82
2000	23/3	3	134/3	11	34	-4	98
1500	25/3	2	136/3	5	37	-1	100
1000	24/3	3	120/3	3	40	2	90
900	21/3	4	161/3	12	26	-12	126
Solvent 1	22/3	3	118/3	2	37		
Solvent 2	24/3	3	135/3	2	36		
Solvent 3	23/3	3	131/3	6	35		
Solvent 4	29/3	4	136/3	7	43		
Mean Solvent Mutant Frequency= 38							
Positive Control - 7,12 Dimethylbenz(a)anthracene (µg/ml)							
5.0	37/3	3	10/3	3	740	705	1
2.5	147/3	9	96/3	5	306	271	48
Solvent 1	22/3	3	126/3	3	35		
Solvent 2	22/3	2	125/3	8	35		
Mean Solvent Mutant Frequency= 35							

[a] - Average # of colonies per plate and # of plates scored

[b] - Mutant frequency (per 10^6 surviving cells) = (Average # TFT colonies / average # VC colonies) x 200

[c] - Induced mutant frequency (per 10^6 surviving cells) = mutant frequency - average mutant frequency of solvent controls

[d] - % total growth = (% suspension growth x % cloning growth) / 100

APPENDIX 3

TABLE 6

CLONING DATA FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS
TREATED WITH IPA Salt of Dicamba
IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION
CONFIRMATORY ASSAY

Test Article Concentration (µg/ml)	Ave #/ TFT Plate[a]	TFT Stand Dev	Ave #/ V.C. Plate[a]	V.C. Stand Dev	Mutant Frequency[b]	Induced Mutant Frequency[c]	% Total Growth[d]
5000	23/3	6	133/3	8	35	-6	66
4500	22/3	2	121/3	13	36	-5	57
4000	29/3	2	145/3	10	40	-1	88
3500	33/3	3	132/3	11	50	9	67
3000	16/3	3	137/3	18	23	-18	81
2500	17/3	3	113/3	3	30	-11	51
2000	23/3	3	109/3	12	42	1	62
1500	25/3	5	115/3	16	43	2	75
1000	31/3	4	158/3	5	39	-2	107
900	26/3	1	120/3	2	43	2	71
Solvent 1	23/3	3	129/3	16	36		
Solvent 2	27/3	4	145/3	5	37		
Solvent 3	35/3	0	133/3	3	53		
Solvent 4	29/3	6	161/3	11	36		
Mean Solvent Mutant Frequency= 41							
Positive Control - Ethyl Methanesulfonate (µl/ml)							
0.50	174/3	8	42/3	4	829	790	12
0.25	161/3	14	71/3	8	454	415	29
Solvent 1	32/3	5	141/3	12	45		
Solvent 2	26/3	4	165/3	1	32		
Mean Solvent Mutant Frequency= 39							

[a] - Average # of colonies per plate and # of plates scored

[b] - Mutant frequency (per 10^6 surviving cells) = (Average # TFT colonies / average # VC colonies) x 200

[c] - Induced mutant frequency (per 10^6 surviving cells) = mutant frequency - average mutant frequency of solvent controls

[d] - % total growth = (% suspension growth x % cloning growth) / 100

APPENDIX 4

TABLE 8

CLONING DATA FOR L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS
TREATED WITH IPA Salt of Dicamba
IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION
CONFIRMATORY ASSAY

Test Article Concentration (µg/ml)	Ave #/ TFT Plate[a]	TFT Stand Dev	Ave #/ V.C. Plate[a]	V.C. Stand Dev	Mutant Frequency[b]	Induced Mutant Frequency[c]	% Total Growth[d]
5000	60/3	3	158/3	13	76	22	49
4500	74/3	7	174/3	7	85	31	66
4000	51/3	6	153/3	11	67	13	67
3500	63/3	3	172/3	23	73	19	96
3000	46/3	8	169/3	13	54	0	85
2500	59/3	4	204/3	11	58	4	114
2000	47/3	1	190/3	14	49	-5	97
1500	53/3	5	203/3	12	52	-2	106
1000	41/3	5	164/3	16	50	-4	88
900	58/3	1	155/3	17	75	21	86
Solvent 1	40/3	2	161/3	11	50		
Solvent 2	47/3	10	167/3	25	56		
Solvent 3	45/3	1	154/3	15	58		
Solvent 4	46/3	6	180/3	6	51		
Mean Solvent Mutant Frequency= 54							
Positive Control - 7,12 Dimethylbenz(a)anthracene (µg/ml)							
5.0	127/3	8	85/3	1	299	247	33
2.5	116/3	9	115/3	15	202	150	68
Solvent 1	38/3	8	151/3	4	50		
Solvent 2	40/3	8	152/3	15	53		
Mean Solvent Mutant Frequency= 52							

[a] - Average # of colonies per plate and # of plates scored

[b] - Mutant frequency (per 10^6 surviving cells) = (Average # TFT colonies / average # VC colonies) x 200

[c] - Induced mutant frequency (per 10^6 surviving cells) = mutant frequency - average mutant frequency of solvent controls

[d] - % total growth = (% suspension growth x % cloning growth) / 100

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Chemical:	**Dicamba, dimethylamine salt**
PC Code:	**029802**
HED File Code	**13000 Tox Reviews**
Memo Date:	**07/26/1996**
File ID:	**TX012000**
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